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December 1985

An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, *Engraulis mordax*

Reuben Lasker (Editor)

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INTRODUCTION: An Egg Production Method for Anchovy Biomass Assessment

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Fishery scientists engaged in estimating the size of free-swimming populations have never had a technique available to them whereby all the parameters could be estimated from a resource survey and where no parameter values need to be assumed. Recognizing the need for a technique of this kind, the staff of the Coastal Fisheries Resources Division of the Southwest Fisheries Center (SWFC) devised an egg production method for anchovy biomass assessment. Previously, anchovy biomass was estimated by approximate methods derived from a long-time series and anchovy larval abundance, which required about 5 mo of shiptime each year to integrate the area under a seasonal spawning curve. One major assumption used in the larval abundance census method is that there is constant proportionality between larval numbers and spawning biomass. This has now proved to be erroneous.

The chief advantages of this egg production method are: 1) it yields an instantaneous estimate of egg production and spawning biomass requiring a single cruise with one or two ships, and 2) each factor in the biomass estimate is formally derived with estimates of precision. The major disadvantage of the method is that eggs are patchier and represent a shorter time period than larvae. This requires that numerous samples be taken to improve precision. Each sample must be small to reduce variance and to limit the time needed to sort out the eggs, and the entire spawning area should be encompassed by the cruise to detect the geographic edge of spawning.

This egg production method is based on an original finding by Moser (1967) that postovulatory follicles can be seen and used to determine time of spawning in rockfish. Hunter and Goldberg (1980) and Hunter and Macewicz (1980), following upon this suggestion, developed criteria for ageing postovulatory follicles in anchovy and hence the frequency of spawning of natural populations. This was confirmed in the SWFC aquarium using Leong's (1971) laboratory breeding anchovies. Thus incidence of females with postovulatory follicles or hydrated eggs could be used as a measure of spawning frequency. Parker's (1980) model, in which all parameters can be estimated, uses an estimate of egg production divided by the product of batch fecundity and the proportion of females in the mature stock, and accounts for the fact that spawning in anchovies is relatively continuous. Estimates of egg production are derived from direct plankton net sampling using a net designed by Smith and modified by Flerx (see Smith et al. 1985). Smith conceived the idea of sampling small numbers of eggs, and guided the procedure at sea each year since its inception, 1980-84, at the SWFC. Lo (1985) developed analytical procedures to estimate the natural mortality and the contagious distribution of the eggs. Moser and Ahlstrom (1985) developed the criteria for staging the eggs.

Besides an estimation of biomass, the application of this technique provides a great deal of information heretofore unobtainable on the natural history of anchovy populations. For example, we now can determine the mortality rates of eggs and early larvae and we have shown that multiple spawners can spawn as many as 20-30 times in a season. The biological rates we measure appear to be much more dynamic than we had supposed; egg mortality has differed greatly from year to year as has fecundity and spawning frequency. Together these new findings have given us an insight we did not have before into the potential for recruitment in fish populations.

It is our belief that the egg production method may also be the technique of choice for determining the spawning biomass of other multiple spawning pelagic fish, particularly clupeoids, (e.g., sardine, anchovy, and menhaden). Thus this volume is intended to be a guide to fishery scientists in applying the method to their own species. We have provided the theoretical basis and described the

operational aspects of the method as we have used it to determine the spawning biomass of the northern anchovy, *Engraulis mordax*, for the last 5 yr in the waters off California and Baja California, Mexico.

There are several important criteria which must be met before this egg production method for biomass estimation can be used. The fish must be a multiple spawner, and its eggs must be pelagic. The eggs must be caught by a plankton net in the upper layers of the ocean without significant losses by extrusion. Spawning and nonspawning adults must be equally available to a trawl or similar sampler. Application of this technique to other fish may require modification to fit the biology of the species and circumstances of sampling and capture.

This manual provides a description of the biomass model and its mathematical parameters; a physical description of the parameters themselves, e.g., eggs and spawning adults; and information on the field methods as we have applied them to determining the spawning biomass of the northern anchovy.

The various steps of the egg production method for determining northern anchovy biomass, as they are done at La Jolla, are charted in Figure 1.

ACKNOWLEDGMENTS

We are indebted to many people for making this work possible and they have been recognized by the SWFC for their individual contributions. Special mention must be made of the officers and crew of the RV *David Starr Jordan* who have aided us every step of the way in making this method a successful one.

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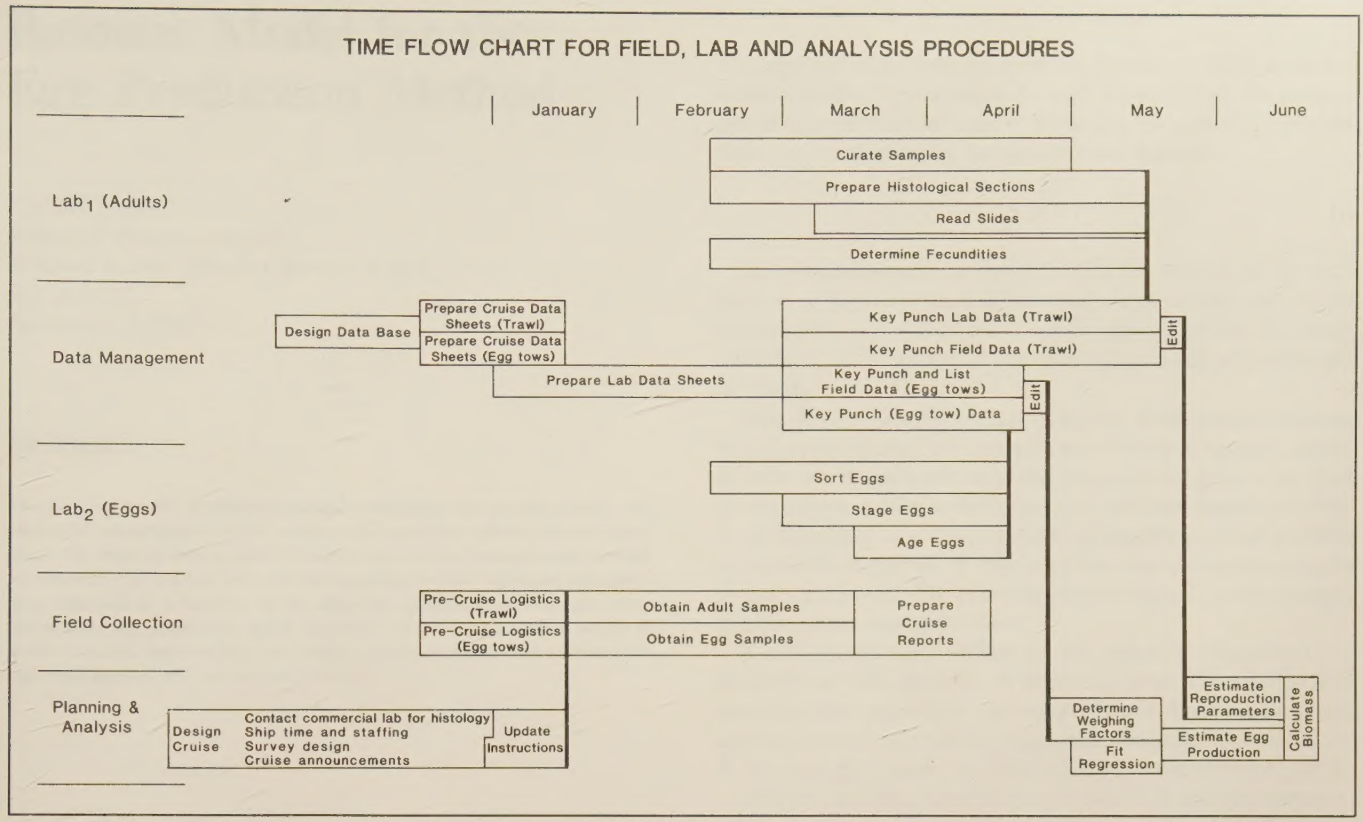

Figure 1.—Time flow chart for field, laboratory, and analysis procedures.

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Biomass Model for the Egg Production Method

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ABSTRACT

The spawning fraction of multiple spawners is estimable when females exhibit morphological characteristics which indicate when spawning will take (or has taken) place. The length of time in which characteristics remain detectable must in itself be estimable and constant over the field sampling interval. Spawning biomass is then estimated as a function of the estimated spawning frequency and other parameters: egg production, batch fecundity, sex ratio, and average weight of mature females. Based on the delta method, approximate variance estimates for spawning biomass are derived and given.

The relationship between the spawning biomass of a fish stock and its production of eggs is easily derived. Simply stated, the production of eggs (P) must be equal to the female biomass that produced those eggs multiplied by the female batch fecundity:

$$P = (B \cdot R') \cdot F'. \quad (1)$$

The female biomass is represented as the product of the total biomass of the entire stock B, both males and females, and R', the portion of the entire stock that is egg-producing females. The batch fecundity F' is the number of eggs spawned per batch per unit weight of female.

Note that R' is not the biomass of females divided by the biomass of males plus females, a simple sex ratio in terms of biomass. Rather R' is the total biomass of females that has produced eggs in a specified period of time divided by the biomass of males and females together. If females spawn more than once during the period of time in which production is measured, R' may be greater than the simple sex ratio in terms of biomass. The converse would be true if, on the average, females spawn less than once.

It is this fundamental definition of R' that allows Equation (1) to be easily derived. However, in its present form, Equation (1) is of practical value only when each mature female spawns once during the time interval over which production is measured, in which case R' becomes the simple sex ratio estimated from biomass, say R.

In its present form, Equation (1) is not useful for multiple spawners, when population spawning appears to be continuous over the period of time over which production is measured. There is no way to relate production of eggs, P, to female biomass that produced those eggs without making an adjustment for spawning frequency.

R', the proportion of female-producing biomass, is composed of two parts: R, the simple biomass-based sex ratio (the biomass of females to that of males plus females) and f, the fraction of females spawning during the time interval,

$$R' = R \cdot f. \quad (2)$$

Parker (1980) documented the above relationship and demonstrated that spawning frequency (f) can be estimated if three conditions are met: 1) Females can be examined for a characteristic which indicates when spawning will or has taken place, 2) the length of time such a characteristic remains detectable is estimable, and 3) the spawning rate (or frequency) remains constant over the sampling interval in which f is estimated.

Under these conditions the spawning fraction (f) is the fraction of females displaying characteristic 1 above, divided by the length of time the characteristic remains detectable. For example, if from a sample of 10 females, 2 display a characteristic which lasts for 1 d and which indicates that spawning will take place in approximately 3 d, then the spawning rate can be expected to be 1/5 in 3 d. Spawning frequency so estimated is additive. For instance, a daily rate can be summed over any length of time, week, year, etc. However, if multiple spawnings occur in the time period, parameter f can exceed unity and is no longer properly a "fraction."

Having developed a condition under which the spawning frequency can be estimated, Parker (1980) rewrote Equation (1) in terms of the simple biomass sex ratio (R) and the spawning frequency (f),

$$P = B \cdot R \cdot f \cdot F'. \quad (3)$$

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Spawning biomass can be estimated directly,

$$B = P/(R \cdot f \cdot F'). \quad (4)$$

Equation (4) is a conventional equation relating spawning biomass to egg production. The estimate of production (P) can be for any time interval, as long as the fraction of females spawning (f) is computed for the same interval. For a species that spawns but once over the time interval in which production is measured, say a year, Equation (4) is still valid, but now greatly simplified since $f = 1$.

Stauffer and Picquelle (1980) modified Equation (4) for the northern anchovy, *Engraulis mordax*, and based the biomass equation on a daily estimate of production and fraction spawning,

$$B = P \cdot A(k \cdot W)/(R \cdot F \cdot S) \quad (5)$$

where B = spawning biomass in metric tons,
P = daily egg production, numbers of eggs produced per 0.05 m^2 per day,
W = average weight of mature females (g),
R = sex ratio, fraction of population that are mature females, by weight (g), as before,
F = batch fecundity, number of eggs spawned per mature females per batch,
S = the fraction of mature females spawning per day,
A = the total survey area (in 0.05 m^2),
k = conversion factor of grams to metric tons.

Stauffer and Picquelle (1980) found that more stable estimates of spawning biomass are achieved if Parker's batch fecundity estimate by weight is replaced by F, eggs per female per batch, and W, the average weight of mature females. Stauffer and Picquelle (1980) demonstrated estimation with an example. A detailed example is given in Picquelle and Stauffer (1985).

Based on the delta method (Seber 1973), Stauffer and Picquelle (1980) show the approximate bias and variance of the biomass estimator to be a function of sample variances and covariances. Bias (b) is given by

$$E[B] = \hat{B} + b \quad (6)$$

where $E[B]$ is the expected value of the biomass and $\hat{B}$ is the estimate from Equation (5). The bias is approximately

$$b \cong \hat{B}(CV(R)^2 + CV(F)^2 + CV(S)^2 + COVS) \quad (7)$$

where CV denotes coefficient of variation, and $COVS$ is the sum of terms involving covariances:

$$\begin{aligned} COVS = {} & COV(PW)/PW - COV(PR)/PR - COV(PF)/PF \\ & - COV(PS)/PS - COV(WR)/WR - COV(WF)/WF \\ & - COV(WS)/WS + COV(RF)/RF + COV(RS)/RS \\ & + COV(SF)/SF. \end{aligned} \quad (8)$$

Ignoring the bias, approximate variance of the estimate is given by

$$Var\ B \cong \hat{B}^2(CV(P)^2 + CV(W)^2 + CV(F)^2 + CV(S)^2 + 2\ COVS) \quad (9)$$

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Parameter Estimation for an Egg Production Method of Northern Anchovy Biomass Assessment

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ABSTRACT

The estimate of daily egg production is derived from the exponential mortality model fit to the egg density data generated by the plankton survey. The specific formulation of the model used in any particular application depends on the availability of computer programs to run the regression analysis. The model also provides an estimate of egg mortality. Stratification and station weighting schemes depend on survey station pattern. Poststratification of plankton stations is recommended for eliminating those plankton stations occupied that lie beyond the spawning area.

The adult spawning parameters for the northern anchovy example are estimated from trawl survey data using the equations for the sample mean and variance from cluster sampling theory. The choice of the most appropriate estimate in any particular application depends on the sample design. The need for stratifying the survey depends on whether the daily population fecundity parameters change in a consistent fashion over the range and duration of the survey.

The estimation of average weight is straightforward except that the weight of females with hydrated ovaries must be adjusted downward to correct for the temporary weight gain from the increased fluid in the ovaries. Since batch fecundity cannot be measured for each female fish, it is estimated for each individual from a regression model of batch fecundity and ovary-free body weight derived from a sample of female fish with hydrated ovaries. The station value for batch fecundity is estimated as the sample mean of the estimated fecundities. The variance of the mean fecundity, however, is adjusted to include the additional variance resulting from the regression estimation.

For the northern anchovy trawl survey, spawning fraction is the proportion of the mature females which have day-1 postovulatory follicles. Evidence from these trawl surveys indicate female fish classed as day-0 spawners are oversampled. The number of mature females per station is adjusted to compensate for this bias. The sex ratio parameter is the fraction of the mature population that is female, based on weight rather than numbers. Sex ratio data are best generated from a second subsample. For these trawl surveys, the sampling bias of day-0 spawning females occurs during the peak hours of the evening spawning period and impacts the estimates of spawning fraction and sex ratio.

INTRODUCTION

The development of equations for estimating egg production parameters is based on statistical procedures that are applicable to survey and sampling designs and that give valid estimates of the parameters and their variances. The purpose of this paper is to present the statistical equations for estimating daily egg production and daily specific[2] fecundity parameters and the associated variance estimates that have been used for the northern anchovy. For convenience, a summary of the parameter values estimated for the northern anchovy from 1980 to 1984 is included in Table 1.

Table 1.—Time series of egg production parameters (1980-84).

Parameters		1980	1981	1982	1983	1984
Daily egg production (10^{12} eggs/d)	PA	26.34	20.96	13.51	17.25	12.98
Average female weight (g)	W	17.44	13.37	18.83	11.20	12.02
Batch fecundity (no. eggs/batch per mature female)	F	7,751	8,329	10,845	5,297	5,485
Spawning fraction (no. spawning females per mature female)	S	0.142	0.106	0.120	0.094	0.160
Sex ratio (no. females/total)	R	0.478	0.501	0.472	0.549	0.582
Daily specific fecundity (no. eggs/g biomass per d)		30.28	33.03	32.53	24.35	42.43
Spawning biomass (10^3 t)	B	870	635	415	652	309

DAILY EGG PRODUCTION

The estimate of daily production of eggs released into the sea, P, by spawning adult fish is derived by regressing the counts of eggs on their age using the exponential mortality model. This model assumes a constant mortality rate.

$$P_{ijk} = P_i e^{(-Zt_{ijk})} + \epsilon_{ijk} \quad (1)$$

where P_{ijk} = the number of eggs in day k age category from station j in stratum i,
t_{ijk} = the age in days measured as the elapsed time from the specified spawning time, t_0, to the time of sampling of station j in stratum i,
P_i = the daily egg production per unit area in stratum i,
Z = the daily rate of instantaneous egg mortality, and
ϵ_{ijk} = the additive error term.

A number of regression procedures can be used to estimate P and Z from the observations of P_{ijk} and t_{ijk} depending on the design of the survey and the availability of statistical computer programs.

First, the need to stratify the plankton survey should be evaluated. Stratification is usually undertaken to reduce the variance of parameter estimates. If more than one major spawning area exists, or if the survey is conducted over a relatively long period such that spawning rates differ among areas or over time, stratification of the

[1]Present addresses: Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, 7600 Sand Point Way NE, Seattle, WA 98115.

[2]Daily specific fecundity = number of eggs produced/gram of biomass per day.

data after the survey would be appropriate. This occurred in the anchovy spawning survey conducted in 1983 (Picquelle and Hewitt 1984).

In addition, the total survey area may be considerably larger than the spawning range. This has been the case for the anchovy resource off the California coast. The anchovy surveys are intentionally designed to sample the full range of anchovy spawning, knowing that in any one year the spawning will occur over a much smaller area within the range. As a result, many stations are beyond the spawning area of anchovies and contribute a large number of stations with zero egg counts, thus inflating the variance of the egg count data set. To reduce the impact of these zero-count stations, the total survey area is poststratified into two strata depending on the presence or absence of eggs in the sample. Stratum 0 contains the stations beyond the geographic area of spawning so that P is zero for stratum 0. The geographic area containing all the anchovy eggs and the few embedded stations with zero egg counts make up stratum 1. In this latter case, the size of the geographic area of stratum 1, A_1, and the number of occupied stations, n_1, become random variables with variances and expected values. The magnitude of this added variance is negligible if n_1 is larger than 100 (Jessen 1978). Poststratification of the stations to eliminate those stations beyond the spawning area is a recommended procedure for estimating egg production.

In addition to stratification, it is desirable to increase the density of plankton stations within the geographic area where adult spawning is expected. Increased sample size in this area will reduce the variance of P for the positive stratum. To correct for differences in station density, the egg count observations by station need to be weighted by a factor proportional to their representative area such that the sum of the station weights in the positive stratum, i=1, equals n_1, the total number of stations in this stratum, i.e.,

$$\sum_{j=1}^{n_1} w_{1j} = n_1 .$$

Since the egg counts in the zero stratum are zero, weighting in that stratum is unnecessary. The preferred statistical technique for estimating parameters P and Z from Equation (1) is weighted nonlinear least squares regression fit to individual egg counts and ages from stations within the positive stratum. The computer program used for the northern anchovy case was BMDPAR (Dixon and Brown 1981).

The stratified estimate of P can be calculated as the weighted average of P_0 and P_1, where P_0 is zero by definition and P_1 is estimated by regression analysis, and the weights are the relative areas of the two stratum, i.e.,

$$P = \frac{A_1}{A} P_1 + \frac{A_0}{A} P_0$$

and the variance, adjusted for postsurvey stratification (Jessen 1978) is

$$Var(P) = (1+\frac{1}{n})(\frac{A_1}{A} Var(P_1) + \frac{A_0}{A} Var(P_0))$$

where A_i = the area of stratum i for each region,
A = $A_1 + A_0$,
n = the total number of observations for the survey,
$Var(P_1)$ = estimated for stratum 1 from the regression analysis, and
$Var(P_0)$ = 0 by definition.

In the event that a computer program for nonlinear regression is not available, the egg mortality model must be linearized so that P and Z can be estimated by linear regression methods. The linear version of the model is

$$\ln(P_{1jk}) = \ln P_1 - Zt_{1jk} + \epsilon_{ijk} .$$

This linear model gives the error structure of ϵ as additive in the transformed expression. This implies a multiplicative error structure in the nonlinear model. The form of the error in either case should be studied by examining the variability about P_{1jk} versus age. This can best be accomplished by examining the residuals between the observed P_{1jk} and those predicted by the model. The antilog estimate of P will be biased. An unbiased estimate can be approximated by

$$P = e^{(\ln P + s^2/2)}$$

where s^2 is the estimated residual variance from the regression analysis, usually denoted by $s_{y \cdot x}{}^2$.

If computer facilities are not readily available, the estimation of P and Z can be further simplified. This simplification involves averaging the egg counts P_{1jk} over time intervals of equal length. A minimum of three time intervals is necessary to estimate the two parameters P and Z. For the anchovy example, five intervals of 12 h were tried. The modified mortality model in this case is[3]

$$\bar{P}_{1k} = P_1 e^{-Zt'_k}(1 - e^{-Z\Delta t})/Z\Delta t$$

where $\bar{P}_{1k}$ = the average number of eggs of age t_k sampled during the time interval (t_k, t_{k+1}),
$t'_k = t_k - t_0$ = the time elapsed (or age) between spawning and the beginning of the time interval, t_k,
t_0 = the midpoint of the daily spawning period, and
Δt = the length of the time interval $(t_{k+1} - t_k)$ over which P_{1jk} are averaged.

The linear form of this model is

$$\ln(P_{1k}) = \ln(P_1(1 - e^{-Z\Delta t})/Z\Delta t) - Zt'_k$$

where the regression coefficients $b_0 = \ln(P_1(1-e^{-Z\Delta t})/Z\Delta t)$, and $b_1 = -Z$. Substituting the value of Z from b_1 into b_0 will provide an estimate of P_1. These estimates of P and Z should be useable in most cases, but their associated variance estimates will not be realistic.

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Station Weighting Alternatives

In sampling theory, several estimates of the population mean are described; the choice of the most appropriate estimate depends on the sample design. Trawl surveys typically consist of a three-stage sampling design: 1) the selection of the trawl stations, 2) the catch of fish at the station, and 3) the selection of the subsample of fish from the catch. The sample design, and hence the estimate, is determined by how each of the stages is executed.

There are two common methods for selecting station locations: random sampling and judgment sampling. Under the random sampling regime, the trawl stations are distributed randomly over the survey area. The exact locations of the stations are determined principally by the requirements of convenient and efficient use of ship time, and by the desire to have stations distributed evenly over the

[3]McCaughran, C. A., Intl. Pacific Halibut Comm., Seattle, WA., pers. commun. May 1981.

survey area. This strategy will produce station locations independent of local fish population densities.

Judgment sampling uses information independent of the trawl samples to place stations where fish abundances are high. This information may consist of a historical account of the distribution of fish based on current sea temperature and salinity data, results from a recent survey, the use of sonar to detect fish schools, or observable evidence of local fish concentrations, such as their spawning products in plankton samples. The resulting distribution of stations will be patchy, with the high densities of stations coinciding with high densities of fish. This strategy approximates the sampling technique of probability proportional to a measure of size.

The second stage of sampling, the catch of fish, is ideally a random sample of fish residing at the station. However, some bias may occur due to net design, the execution of the trawl, fish behavior, and other variables. In addition to striving for a random sample, it is also advantageous if the catch size is proportional to the number of fish at the station. This is valuable information which may be incorporated into the estimate, but this situation holds only for certain species and sampling methods.

Fish are subsequently sampled randomly from the catch. The subsample size is usually constant, but may vary with the catch size to produce a self-weighting estimate for some of the estimates presented below.

Based on this sampling structure, many specific sample designs and corresponding estimates of the population mean may be considered. The following estimates all assume that the total population size is very large and the finite population correction is approximately 1. Under these conditions, the within-subsample contribution to variance disappears because this quantity is multiplied by $1/Nn$ which is approximately 0.

If stations are selected randomly, and catch size is unrelated to fish abundance, then each station should receive equal weight and equal subsample sizes should be attempted. In this case the appropriate estimate is (Cochran 1977)

$$\bar{\bar{y}} = \frac{\sum_{i=1}^{n} \bar{y}_i}{n} \quad \text{and} \quad \hat{Var}(\bar{\bar{y}}) = \sum_{i=1}^{n} \frac{(\bar{y}_i - \bar{\bar{y}})^2}{n\,(n-1)} \tag{2}$$

where $\bar{\bar{y}}$ = the estimate of the population mean,

n = the number of stations,

$\bar{y}_i = \sum_{j=1}^{m_i} \frac{y_{ij}}{m_i}$ = the mean of the ith station, and

m_i = the number of fish subsampled from the ith catch.

This estimate is a biased estimate of the true population mean and this bias does not necessarily get small as n gets large. The estimate is self-weighting if m_i is constant.

A better estimate exists for the case of random station selection if the catch size is proportional to the abundance of fish at the station. This is the ratio-to-size estimate:

$$\bar{\bar{y}} = \frac{\sum_{i=1}^{n} M_i \bar{y}_i}{\sum_{i=1}^{n} M_i} \quad \text{and} \quad \hat{Var}(\bar{\bar{y}}) = \frac{\sum_{i=1}^{n} M_i^2 (\bar{y}_i - \bar{\bar{y}})^2}{\left(\sum_{i=1}^{n} \frac{M_i}{n}\right)^2 n\,(n-1)} \tag{3}$$

where M_i is the total number of the target fish species caught in the ith trawl. This estimate is biased because it is a ratio of two random variables, but the bias is small and gets smaller as n gets larger. This estimate is self-weighting when the subsampling fraction m_i/M_i is constant.

Under judgment sampling, the attempt is made to sample with probability proportional to size. If this can be accomplished, or if confidence is high that this situation is closely approximated, then the unbiased estimates of the population mean and the variance are the same as Equation (2). This estimate is self-weighting when m_i is constant.

If, instead, the stations are selected with probability proportional to u_i, a measure of size, and the catch size is proportional to the population size at the station, then the appropriate estimate is

$$\bar{\bar{y}} = \frac{1}{M_0 n} \sum_{i=1}^{n} \frac{M_i \bar{y}_i}{u_i} \quad \text{and} \quad \hat{Var}(\bar{\bar{y}}) = \frac{\sum_{i=1}^{n} \left(\frac{M_i \bar{y}_i}{u_i M_0} - \bar{\bar{y}} \right)^2}{n\,(n-1)} \tag{4}$$

where M_0 is the total population size. This estimate is self-weighting if $nu_i m_i / M_i$ is constant. However, this estimate is not very useful because M_0, the total population size, is rarely known, and the estimates of station sizes, u_i, can rarely be enumerated.

A modification of Equation (2) is used for the trawl survey for the northern anchovy. As mentioned earlier, this estimate is unbiased if sampling with probability proportional to size. The information used to detect high concentrations of anchovies is the occurrence of anchovy spawning products in the plankton samples taken concurrently, and the presence of apparent schools on the sonar. Both of these factors are good indicators of local concentrations of anchovies, and the resulting sample design is assumed to be a good approximation of sampling with probability proportional to size, justifying the use of Equation (2).

Equal subsample sizes are attempted, but occasionally a station will produce a very small catch or a catch with very few mature females (mostly males or mostly immature fish). Both situations will result in a small subsample, as most of the parameters to be estimated are for mature females. This occurrence is interpreted as meaning that an error in judgment sampling has been made. The actual size of the station, based on mature females, is much smaller than was estimated at the time the trawl station was selected. Hence, the probability of selecting that station should be adjusted *a posteriori* to reflect the actual size of the station. This is accomplished by giving these stations less weight in the estimate; each station is weighted by its subsample size, m_i. Thus, Equation (2) is modified to produce the following estimate:

$$\bar{\bar{y}} = \frac{\sum_{i=1}^{n} m_i \bar{y}_i}{\sum_{i=1}^{n} m_i} \quad \text{and} \quad \hat{Var}(\bar{\bar{y}}) = \frac{\sum_{i=1}^{n} m_i^2 (\bar{y}_i - \bar{\bar{y}})^2}{\left(\sum_{i=1}^{n} \frac{m_i}{n}\right)^2 n\,(n-1)} \tag{5}$$

If m_i is constant, this estimate simplifies to Equation (2). This estimate is biased because it is the ratio of two random variables, $m_i \bar{y}_i$ and m_i. However, the bias is of the order $1/n$, so that the bias gets smaller as n gets larger.

Trawl Survey Stratification

The egg production model assumes that the parameters in the model are constant over the range and duration of the survey. If this assumption is violated, the survey should be divided into regions or time spans within which the parameters are constant. The biomass is then estimated separately for each section of the survey and then summed to produce the total biomass estimate.

An example of this situation is the Spring 1983 anchovy spawning biomass survey. Two parameters of the daily specific fecundity varied significantly with geographic regions, female weight decreased from north to south, and spawning fraction increased from north to south.

The survey area was divided into three regions: north, bight, and south (Figs. 1, 2). Figure 3 illustrates the frequency distribution for female weight by region; the average female weight for the southern region was significantly smaller than the average weights for both the bight and north regions. The pattern for spawning fraction was the opposite (Fig. 4) with the estimate for the north region being significantly smaller than the estimates for the bight and south regions.

Table 2 presents the parameter estimates and biomass estimates for each region. Each of the population fecundity estimates, W, S, F, and R, was estimated separately for each region. It was impossible to estimate P_0, egg production, independently for each region because of the small sample sizes per region. Instead, the slope of the mortality curve, Z, was assumed to be constant for the entire survey and hence was estimated using all the data. The intercept of the mortality curve, P_0, was allowed to vary between regions and was estimated by fitting a separate but parallel mortality curve to each region while holding the mortality estimate, Z, fixed at the value previously estimated.

The total biomass estimate is simply the sum of the regional biomass estimates, and the total variance estimate is also just the sum of the regional variances. This variance estimate ignores any covariance terms between regions, which is probably trivially small because all parameters were estimated using separate and independent data for each region, except for the mortality, Z, whose contribution to covariance is probably slight.

Table 2.—Estimates of egg production parameters and spawning biomass by region for 1983. Coefficient of variation indicated by parentheses.

Parameters		North	Bight	South	Total
Daily egg production (no. eggs/0.05 m^2 per d)	P	1.62 (0.671)	7.28 (0.0751)	5.06 (0.332)	
Area of region (10^{12} 0.05 m^2)	A	0.420	1.33	1.36	
Average female weight (g)	W	12.9 (0.121)	11.2 (0.0705)	9.63 (0.0385)	
Batch fecundity (no. eggs/batch per mature female)	F	6,285 (0.140)	5,295 (0.0882)	4,423 (0.0570)	
Spawning fraction (no. spawning females per mature female)	S	0.0346 (0.563)	0.103 (0.174)	0.126 (0.237)	
Sex ratio (no. females/total)	R	0.523 (0.0949)	0.559 (0.0736)	0.549 (0.128)	
Spawning biomass (10^3 t)	B	77.5 (0.897)	358 (0.214)	216 (0.419)	652 (0.211)

Parameter Estimation

The parameters of the daily population fecundity are all estimated from samples of anchovies collected on a midwater trawl survey. These parameters, female weight (W), batch fecundity (F), spawning fraction (S), and sex ratio (R), and their variances, are estimated using Equation (5) developed previously:

$$\bar{\bar{y}} = \frac{\sum_{i=1}^{n} m_i \bar{y}_i}{\sum_{i=1}^{n} m_i} \quad \text{and} \quad \hat{Var}(\bar{\bar{y}}) = \frac{\sum_{i=1}^{n} m_i^2 (\bar{y}_i - \bar{\bar{y}})^2}{\left(\sum_{i=1}^{n} \frac{m_i}{n}\right)^2 n(n-1)}$$

where n = the number of trawls,

m_i = the number of fish subsampled from each trawl,

$\bar{y}_i = \sum_{j=1}^{m_i} y_{ij}/m_i$ = the average value for the ith trawl, and

y_{ij} = the observed value for the jth fish sampled from the ith trawl.

Female weight is estimated from a fixed subsample size of mature females. The subsample size has ranged from 15 to 25 mature females for the surveys taken from 1980 through 1983; however, the targeted subsample size is not always realized, due to very small catches, a high proportion of immature fish, or a high proportion of males. The y_{ij} in Equation (5) is the whole body weight of the jth mature females sampled from the ith trawl (W_{ij}). This observed weight is adjusted downward for those females whose ovaries contain hydrated eggs because their body weight is temporarily inflated due to water retention. This adjusted weight ($\hat{W}_{ij}$) is estimated from a linear regression of whole body weight regressed on ovary-free weight (W_{ij}*) which is fit only to those females that do not have hydrated eggs.

$$\hat{W}_{ij} = \hat{\alpha} + \hat{\beta} W_{ij}^* \tag{6}$$

The observed frequency distribution of the average female weight per trawl is usually symmetrical although there may occur a hint of bimodality if there is a large 1-yr-old year class. The weights within each trawl tend to be homogeneous, suggesting that the anchovy schools are homogeneous with regard to weight.

Batch fecundity can be observed only for those females whose ovaries contain hydrated eggs. There is a high correlation between the number of eggs per batch and the ovary-free body weight. This relationship is used to estimate batch fecundity for the same mature females used to estimate female weight.

The sample of hydrated females is collected throughout the trawl survey and the number of eggs per batch (F_{ij}) and ovary-free weight (W_{ij}*) are recorded for each of these females. This data are used to fit a model regressing batch fecundity on ovary-free weight

$$F_{ij} = \alpha + \beta W_{ij}^* + \epsilon_{ij}. \tag{7}$$

A linear regression has explained the data satisfactorily in previous surveys, although a curvilinear model should be considered depending on the shape of the data.

Using this regression, batch fecundity is estimated for each mature female subsampled. The accuracy of the estimated batch fecundity will be improved if the distribution of weights for the sample of hydrated females used to fit the regression is similar to the distribution of weights for the total sample of females.

Equation (5) is used to estimate average batch fecundity where the y_{ij} are in this case the estimates $\hat{F}_{ij}$. However, there is an added source of variance that should be included because the $\hat{F}_{ij}$ are not observed directly but are estimates with their own associated variance. Thus, the estimate of variance is adjusted to include this additional variance (Draper and Smith 1966):

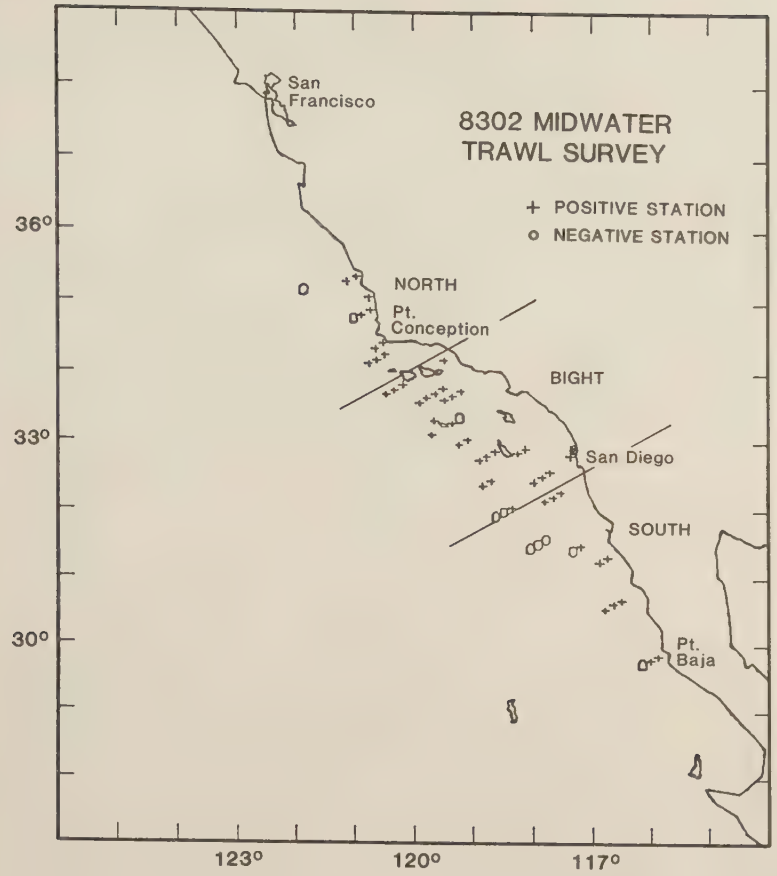

Figure 1.—Geographic distribution of trawl stations and positive trawls within each region.

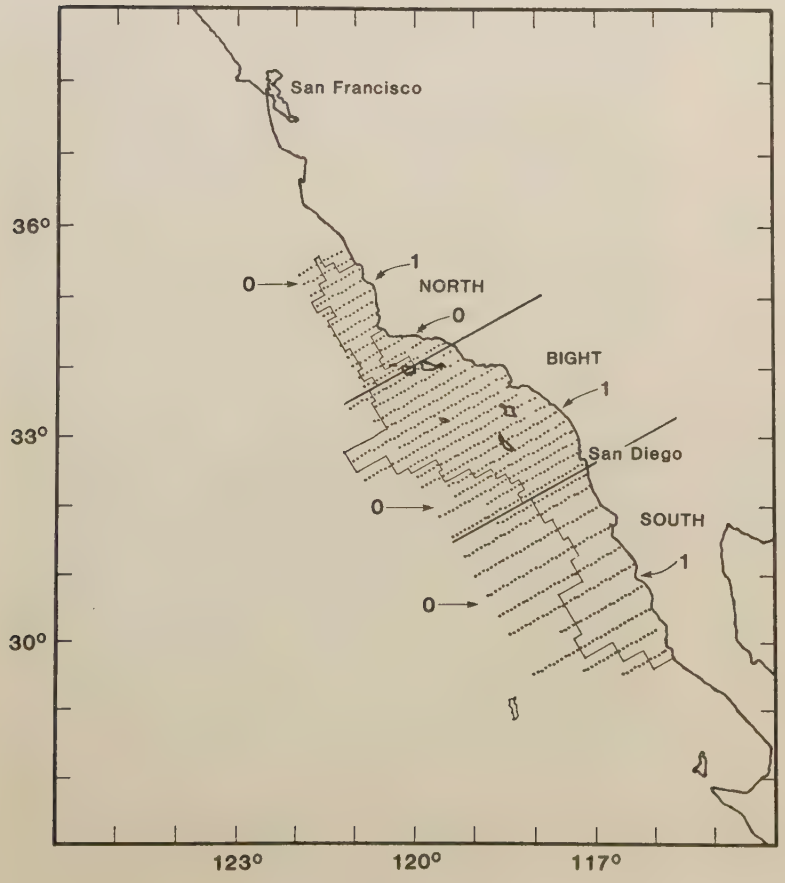

Figure 2.—Subdivision of CalVET survey into strata (0 = beyond the range of anchovy spawning; 1 = within the range of anchovy spawning) and regions (North, Bight, and South).

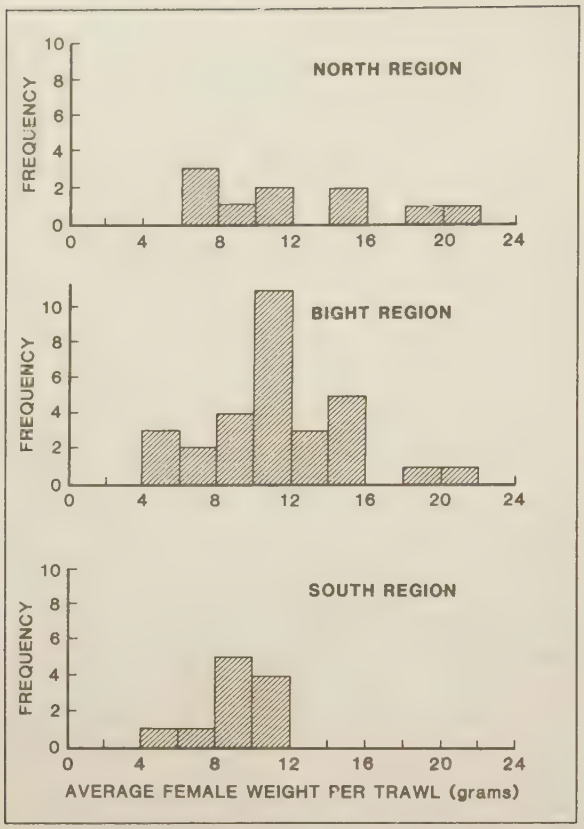

Figure 3.—Frequency distribution for average whole-body weight of mature females in grams for each region.

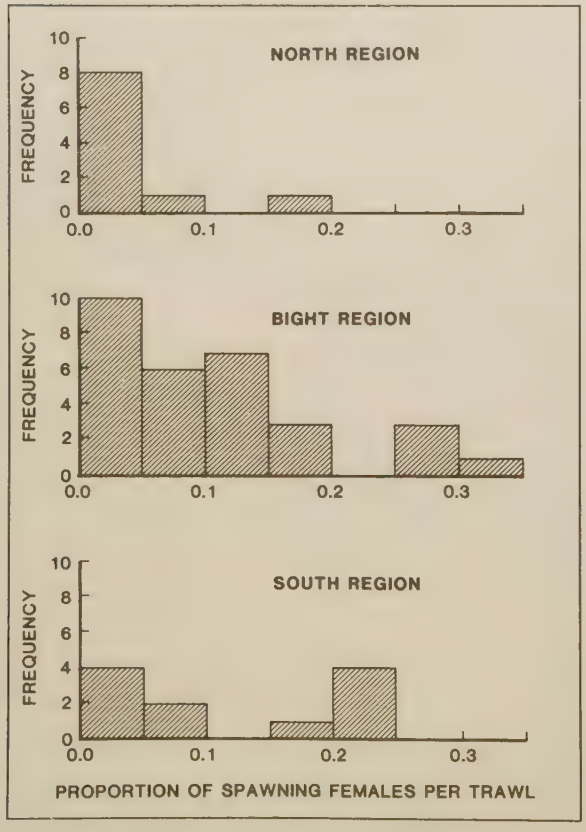

Figure 4.—Frequency distributions of spawning fractions for each region.

$$\hat{Var}(\bar{\bar{F}}) = \frac{\sum_{i=1}^{n} m_i^2 \left[\frac{(\bar{F}_i - \bar{\bar{F}})^2}{n-1} + \frac{s_h^2}{n_h} + (\bar{W}_i^* - \bar{W}_h^*)\, \hat{Var}(\hat{\beta}) \right]}{\left(\sum_{i=1}^{n} \frac{m_i}{n} \right)^2 n} \quad (8)$$

where $\bar{\bar{F}}$ = the estimate of batch fecundity for the whole population of mature females,

$\bar{F}_i$ = the average batch fecundity for the ith trawl,

$\bar{F}_i = \sum_{j=1}^{m_i} \hat{F}_{ij}/m_i$ where $\hat{F}_{ij}$ is the estimated batch fecundity for the jth female in the ith subsample,

s_h^2 = the variance about the regression (Equation (7)),

n_h = the number of hydrated females used to fit the regression (Equation (7)),

$\bar{W}_i^*$ = the average ovary-free weight for the ith trawl,

$\bar{W}_h^*$ = the average ovary-free weight for the n_h hydrated females, and

$\hat{Var}(\hat{\beta})$ = the variance of the slope of the regression (Equation (7)).

Spawning fraction is estimated using Equation (5) by setting $\bar{y}_i$ equal to the proportion of mature females in the ith trawl which have been classified as having day-1 postovulatory follicles (day-1 spawners). Thus, $\bar{\bar{y}}$ estimates the fraction of mature females in the population that are day-1 spawners, which is a measure of the fraction of mature females which are spawning on any given day.

Spawning fraction can also be estimated by the fraction of females classified as day-0 spawners. However, this fraction has been consistently higher than the fraction of day-1 spawners. Past experience has shown that using day-0 spawners may produce a biased estimate, at least for northern anchovy sampled by a trawl survey. Evidence of this conclusion will be presented in a later section.

If day-0 spawners are indeed oversampled, then day-1 spawners are undersampled. The sample of mature females from each catch may be grouped into three categories: Day-0 spawners (m_i^0); day-1 spawners (m_i^1); and day 2+ spawners (m_i^2). For a fixed subsample size, if one group is over-represented then the other two groups are under-represented. This is corrected by adjusting m_i to reflect what subsample size would have included the observed number of day-1 spawners if day-0 had not been oversampled. The number of day-0 spawners included in m_i is replaced by the observed number of day-1 spawners, since day-0 and day-1 spawners should be equal, on average, because they both measure the number of females spawning during a 24-h period. Thus, the m_i in Equation (5) is replaced by

$$m_i^* = 2m_i^1 + m_i^2 . \quad (9)$$

The average value of m_i^* will be smaller than the average m_i, and the resulting estimate of spawning fraction based on day-1 spawners will be larger to compensate for the bias in sampling day-0 spawners.

The parameter sex ratio is the fraction of the mature population that is female, based on weight rather than numbers. Equation (5) is again used, where m_i is the weight of the subsample rather than number, and $\bar{y}_i$ is the fraction of the subsample weight that is attributable to female fish. Both mature and immature fish are included in the estimate because it is impossible to distinguish between mature and immature males. It is assumed that sex ratio by weight is the same for both mature and immature fish.

To save effort in preserving and weighing individual fish, $\bar{y}_i$ and m_i are estimated rather than measured directly. A fixed number of fish are subsampled from each trawl and the numbers of females and males are recorded. The average weight for each sex is estimated

for each trawl from a smaller fixed subsample of each sex. The total weight of each sex in the subsample is estimated by multiplying the observed number of fish of that sex by its average weight.

$$\hat{W}_i^F = m_i^F \cdot \overline{W}_i^F$$
$$\hat{W}_i^M = m_i^M \cdot \overline{W}_i^M \quad (10)$$

where $\hat{W}_i^k$ = the total estimated weight of the kth sex in the ith subsample,
m_i^k = the number of fish of the kth sex in the ith subsample, and
$\overline{W}_i^k$ = the average weight of the kth sex in the ith subsample.

Then m_i is estimated by the sum of the estimated total weight of males plus the estimated total weight of females,

$$\hat{m}_i = \hat{W}_i^F + \hat{W}_i^M \quad (11)$$

and $\overline{y}_i$ is the estimated total weight of females divided by m_i

$$\overline{y}_i = \hat{W}_i^F / \hat{m}_i \cdot \quad (12)$$

The parameters W_i^F and W_i^M are estimated with little error because the weights of fish within a trawl catch are quite homogeneous. Thus the added variance in estimating sex ratio, due to the fact that $\overline{y}_i$ and m_i are estimated rather than observed, is assumed to be trivial.

Sampling Bias

Spawning fraction may be estimated by either the number of day-0 spawners or the number of day-1 spawners, as each is an estimate of the number of females spawning on any given day. However, as mentioned earlier, the proportion of day-0 spawners is consistently larger than the proportion of day-1 spawners. A hypothesis on the mechanism causing this result is that females who are actively spawning are more vulnerable to capture by a midwater trawl. This hypothesis is supported by the observation that the catch of spawning females (day-0) increases significantly during the hours of the evening when spawning takes place.

This phenomenon is illustrated by Figure 5. This bar chart was calculated using data from the trawl surveys conducted from 1978 to 1980. Day-0 spawners are composed of three types of females: Females whose ovaries contain hydrated eggs which will be spawned later that evening (represented by an open bar); females whose ovaries contain postovulatory follicles indicating they have spawned earlier that evening (represented by the bar area with horizontal lines); and females whose ovaries contain both hydrated eggs and postovulatory follicles indicating they were caught during spawning (represented by vertical lines in the bars). The predominant hours of spawning are 2100-2359, when the highest number of females are caught with both hydrated eggs and postovulatory follicles in their ovaries. This is also the time period when the proportion of day-0 spawners in the catch is highest.

It should also be noted that the proportion of day-0 spawners decreases after 0300. This can be more easily seen in Figure 6, which shows the proportion of day-0 spawners by hour for each year, and all years combined. Figure 7 shows that the proportion of day-1 spawners also drops off after 0300. This is explained by an increased error rate in classifying ovaries as to the date of spawning (day-0, day-1, or later), and in subsequent years trawl catches were not made after 0300 to avoid this problem.

If one ignores the points corresponding to 0300 and later, then

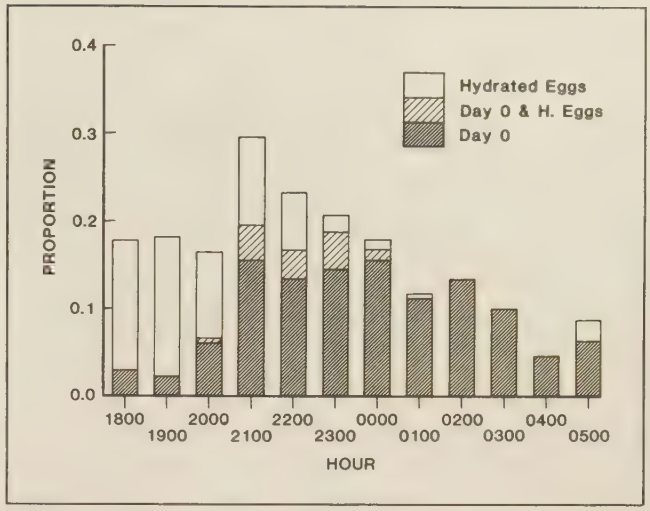

Figure 5.—Breakdown of day-0 females into ovarian categories by time of sampling.

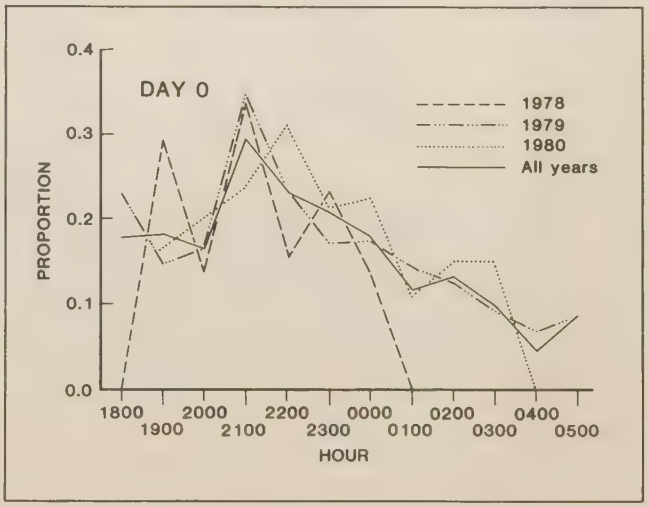

Figure 6.—Proportion of day-0 spawning females by hour for trawl surveys conducted from 1978 to 1980.

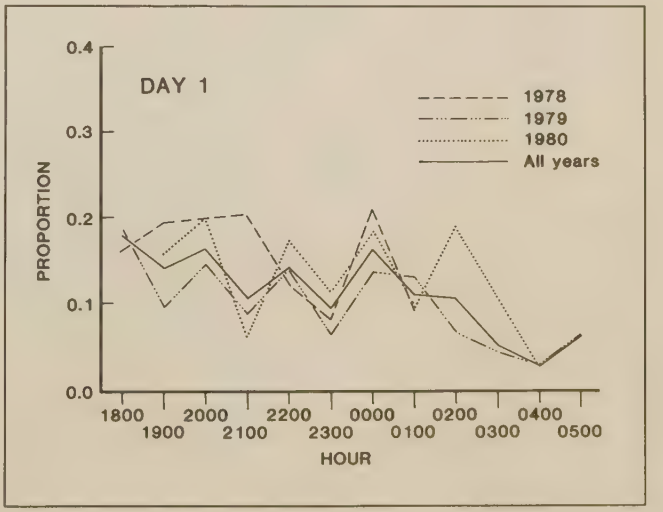

Figure 7.—Proportion of day-1 spawning females by hour for trawl surveys conducted from 1978 to 1980.

a comparison of Figures 6 and 7 shows that the plot of day-1 spawners is quite flat over time, while day-0 spawners exhibit a definite peak at 2100-2359. The comparison is highlighted in Figure 8. The proportion of day-0 females is plotted against the proportion of day-1 females for each hour, and 1-standard-error bars are drawn for day-1 proportions. The diagonal line shows the values for which the proportions of day-0 and day-1 spawners are equal. The obvious outlier points (i.e., those points furthest from the day 0=day 1 line) correspond to the time 2100-2359.

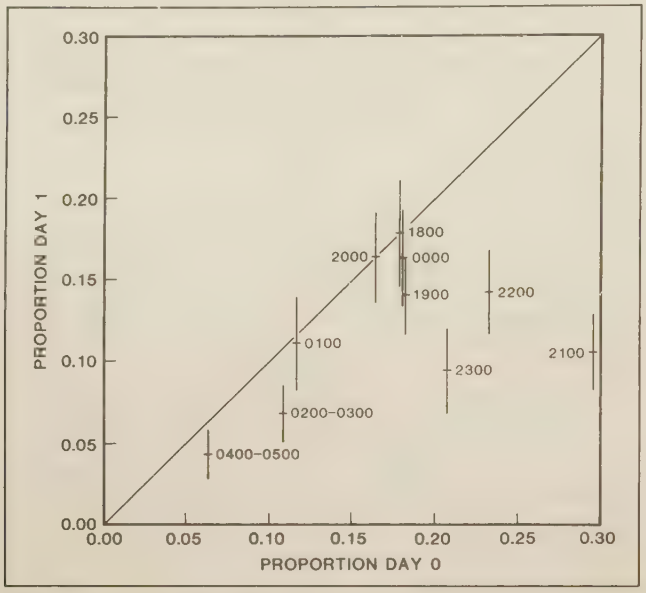

Figure 8.—Day-1 vs. day-0 females for each hook with standard error bars for day-1 females.

The effect of time on the proportion of day-0 and day-1 females in the catch can be quantified using contingency table analysis. Table 3 shows the number of females from the 1978-80 trawl survey samples, by spawning condition and time of their capture. Parentheses contain the expected number of females under the hypothesis that there is no interaction between time and spawning condition. The contribution to the χ^2 statistic by the cell is in brackets. The resulting χ^2 statistic ($\chi^2 = 38.85$, df = 16) is highly significant ($P = 0.05$), thus rejecting the hypothesis of no interaction between time and spawning condition. Examination of the individual cell's contribution to the test statistic shows that the largest deviations are due to the hour 2100-2159. Omitting this hour from the analysis produces Table 4 and a nonsignificant ($P = 0.05$) test statistic ($\chi^2 = 20.51$, df = 14). Therefore, if the hour 2100-2159 is omitted, there is no significant relationship between time and spawning condition. Based on this analysis, the conclusion is made that day-0 spawners are sampled with bias during the peak hours of spawning.

A similar scenario has been discovered for sex ratio and time. The proportion of females declines radically during the time period 2300-2359. A contingency table analysis (Table 5) shows that there is a significant interaction between time and sex ratio ($\chi^2 = 126.44$, df = 8); an examination of the individual cells shows a large deviation during time 2300-2359. When this time period is removed (Table 6), there is no significant interaction ($\chi^2 = 10.63$, df = 7). Apparently, there is a sample bias also occurring for sex ratio during the hours when spawning activity is greatest.

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Table 3.—Contingency table analysis of spawning condition by time. Each cell contains the observed count, the expected count in parentheses, and the contribution to the χ^2 statistic in brackets.

	Time (h)									
Spawning condition	1800-1859	1900-1959	2000-2059	2100-2159	2200-2259	2300-2359	0000-0059	0100-0159	0200-0259	Total
Day-0	25	42	30	67	49	30	29	19	9	300
	(27.5)	(45.3)	(35.9)	(44.5)	(41.4)	(28.4)	(31.8)	(32.0)	(13.3)	
	[0.22]	[0.24]	[0.96]	[11.36]	[1.41]	[0.09]	[0.24]	[5.26]	[1.41]	
Day-1	25	31	30	19	27	12	26	18	7	195
	(17.8)	(29.4)	(23.3)	(28.9)	(26.9)	(18.5)	(20.6)	(20.8)	(8.7)	
	[2.87]	[0.08]	[1.91]	[3.41]	[0.00]	[2.27]	[1.39]	[0.37]	[0.32]	
Day-2+	90	158	123	141	135	103	107	126	52	1,035
	(94.7)	(156.3)	(123.8)	(153.6)	(142.7)	(98.1)	(109.6)	(110.3)	(46.0)	
	[0.23]	[0.02]	[0.01]	[1.03]	[0.42]	[0.25]	[0.06]	[2.25]	[0.78]	
Total	140	231	183	227	211	145	162	163	68	1,530

$\chi^2 = 38.85$, df = 16
$\chi^2_{16}(0.95) = 26.3$

Table 4.—Contingency table analysis of spawning condition by time, in which the hour 2100-2159 has been omitted. Each cell contains the observed count, the expected count in parentheses, and the contribution to the χ^2 statistic in brackets.

	Time (h)								
Spawning condition	1800-1859	1900-1959	2000-2059	2200-2259	2300-2359	0000-0059	0100-0159	0200-0259	Total
Day-0	25	42	30	49	30	29	19	9	233
	(25.0)	(41.3)	(32.7)	(37.7)	(25.9)	(29.0)	(29.1)	(12.2)	
	[0.00]	[0.01]	[0.23]	[3.37]	[0.64]	[0.00]	[3.53]	[0.82]	
Day-1	25	31	30	27	12	26	18	7	176
	(18.9)	(31.2)	(24.7)	(28.5)	(19.6)	(21.9)	(22.0)	(9.2)	
	[1.96]	[0.00]	[1.13]	[0.08]	[2.94]	[0.78]	[0.73]	[0.52]	
Day-2+	90	158	123	135	103	107	126	52	894
	(96.1)	(158.5)	(125.6)	(144.8)	(99.5)	(111.1)	(111.8)	(46.7)	
	[0.38]	[0.00]	[0.05]	[0.66]	[0.12]	[0.15]	[1.79]	[0.61]	
Total	140	231	183	211	145	162	163	68	1,303

$\chi^2 = 20.51$, df = 14
$\chi^2_{14}(0.95) = 23.7$

Table 5.—Contingency table analysis of sex ratio by time. Each cell contains the observed count, the expected count in parentheses, and the contribution to the χ^2 statistic in brackets.

	Time (h)									
Sex	1800-1859	1900-1959	2000-2059	2100-2159	2200-2259	2300-2359	0000-0059	0100-0159	0200-0259	Total
Female	153	349	189	271	227	189	163	187	71	1,799
	(131.2)	(331.2)	(160.3)	(268.3)	(198.7)	(321.9)	(154.4)	(175.5)	(57.4)	
	[3.62]	[0.96]	[5.13]	[0.03]	[4.03]	[54.88]	[0.48]	[0.75]	[3.23]	
Male	158	436	191	365	244	574	203	229	65	2,465
	(179.8)	(453.8)	(219.7)	(367.7)	(272.3)	(441.1)	(211.6)	(240.5)	(78.6)	
	[2.64]	[0.70]	[3.74]	[0.02]	[2.94]	[40.05]	[0.35]	[0.55]	[2.36]	
Total	311	785	380	636	471	763	366	416	136	4,264

$\chi^2 = 126.44$, df = 8
$\chi^2_8(0.95) = 15.5$

Table 6.—Contingency table analysis of sex ratio by time, in which the hour 2300-2359 has been omitted. Each cell contains the observed count, the expected count in parentheses, and the contribution to the χ^2 statistic in brackets.

	Time (h)								
Sex	1800-1859	1900-1959	2000-2059	2100-2159	2200-2259	0000-0059	0100-0159	0200-0259	Total
Female	153	349	189	271	227	163	187	71	1,610
	(143.0)	(361.0)	(174.8)	(292.5)	(216.6)	(168.3)	(191.3)	(62.5)	
	[0.70]	[0.40]	[1.16]	[1.58]	[0.50]	[0.17]	[0.10]	[1.14]	
Male	158	436	191	365	244	203	229	65	1,891
	(168.0)	(424.0)	(205.2)	(343.5)	(254.4)	(197.7)	(224.7)	(73.5)	
	[0.59]	[0.34]	[0.99]	[1.34]	[0.43]	[0.14]	[0.08]	[0.97]	
Total	311	785	380	636	471	366	416	136	3,501

$\chi^2 = 10.63$, df = 7
$\chi^2_7(0.95) = 14.1$

Sea Survey Design and Analysis for an Egg Production Method of Anchovy Biomass Assessment

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ABSTRACT

A sea survey of spawning habitat is described for the northern anchovy, *Engraulis mordax*. The central population occurs within a permanent gyral circulation off southern California and in the adjacent nearshore and main branches of the California Current. The geographic scope of spawning is maximal in winter and spring, and its extent appears to be a function of population biomass.

About 8 hours after the onset of spawning it becomes possible to obtain effective samples: adult data can be used for about a day and egg data for a little more than 2 days to estimate vital rates, such as egg production and mortality. The spawning process is so patchy and dynamic that it has not yet been possible to gather useful data on adults or eggs during the spawning interval.

The population egg production rate is determined by a survey using about 1,000 vertical plankton tows of 3.5 m^3 between 70 m depth and the surface. The array of samples is intended to be representative and inclusive of the entire spawning area. Observations of the number of eggs per sample represent a contagious distribution (patchy), and the assumptions necessary for a "normal" or "log-normal" model are not met. The assumption of independence of sample parameters (mean and variance) is not supported with either model. The probability distribution most closely approximated is the "negative binomial." The parameters of that distribution change with the age of the egg.

The Southwest Fisheries Center Egg Production Method was initiated with much historical, geographic, and biological data obtained in the California Cooperative Oceanic Fisheries Investigations (CalCOFI). It should be possible to initiate an egg production method on other species in other regions without this vast time-series of data. Preliminary laboratory work, field surveys, and analyses are described for research at other temperatures and for the diagnosis of egg production for species which spawn at all times of day rather than nocturnally. The geographic limits of spawning should initially be described from oblique plankton tows filtering larger volumes of water (500-1,000 m^3). Also, differences in spawning patch intensity may require observations of more than the 3.5 m^3 which is adequate for the anchovy in this region.

THE SPAWNING PROCESS

Spawning Area

The northern anchovy, *Engraulis mordax*, spawns within the regional eastern boundary current, the California Current system. Figure 1 is a series of overplots of all samples of anchovy eggs collected from 1951 to 1981. These serve to describe the maximum spatial distribution of spawning in each bimonthly period. Spawning occurs primarily nearshore of the main branch (Hickey 1979) of the California Current. The main branch of the California Current is 300 km off Washington and Oregon (lat. 45°N), 430 km off Cape Mendocino (lat. 39°N), 270 km off Point Conception (lat. 34°N), 240 km off northern Baja California (lat. 30°N), and 200 km off Cape San Lazaro (lat. 25°N).

The boundaries of the main branch of the California Current shift in position and are indistinct at every season. The 1950-78 average in a section normal to the coast at lat. 32°N reveals the three branches of the California Current postulated by Hickey (1979) (Lynn et al. 1982). The speeds of the surface currents at the cores of these three zones are given in Table 1. It may be inferred from Figure 1 that the nearshore zone of the California Current system is the most consistent spawning site of the northern anchovy and that virtually no spawning takes place in the outer zone or in the main branch at the season of heaviest southward flow.

Table 1.—Zones of the California Current.

	Outer		Main		Inner	
Month	u[1]	d[2]	u	d	u	d
January	[3]+3.5	600	− 6.9	400	3.5	100
April	−5.2	500	− 6.9	250	−5.2	50
July	−3.5	700	−12.0	250	6.9	100
October	−2.6	700	− 6.1	400	5.2	100

[1]u is the current speed at the core in kilometers/day.
[2]d is the distance of the core from the coast at CalCOFI line 90 (see Lynn et al. 1982) in kilometers
[3]indicates Equatorward flow.

In addition to the large-scale features, there are local environmental events which appear to influence the pelagic spawning population. Temperature (Lasker et al. 1981; Fiedler 1983) and surface chlorophyll concentration estimated from analysis of satellite infrared images (Pelaez and Guan 1982; Fiedler 1983) appear to be important in fine-scale distributions. In the short period in which satellite image analysis has been possible, 0.2 mg/m^3 appears to be a lower limit of chlorophyll in which anchovy spawning takes place (Fiedler 1983). There also appears to be some diminution of incidence of eggs at temperatures below 13.5°C (Fiedler 1983) although temperatures as low as 11°C are not lethal for anchovy eggs. It also appears that a certain amount of stability in terms of mixing (Lasker 1975; Bakun and Parrish 1982; Smith and Lasker 1978) and absence of offshore and southerly transport (Hewitt and Methot 1982; Power 1983) are favorable to the establishment and maintenance of spawning areas.

Population size also appears to control the spawning area (Ahlstrom 1965; MacCall 1983). There is anchovy spawning further offshore and north when the biomass is large, but the spawning area appears to contract toward the Los Angeles Bight when the biomass is smaller. A simple description of this (MacCall 1983) would be that a 100,000-ton spawning biomass would extend off-

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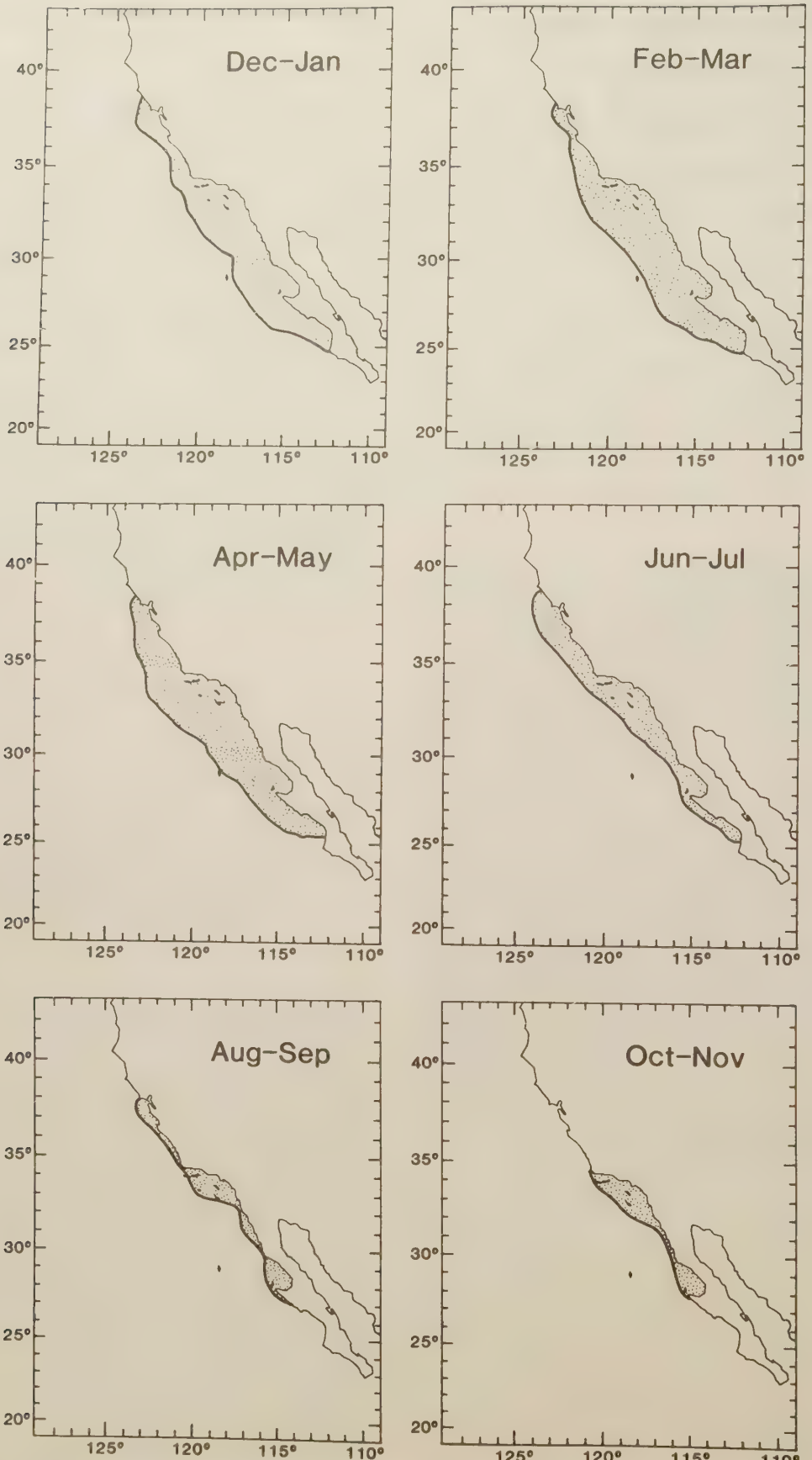

Figure 1.—Long-term spawning range of the northern anchovy as estimated from approximately 30,000 observations of anchovy eggs taken from a larger area between 1951 and 1983. For maps of all observations between 1951 and 1979 consult CalCOFI Atlases 9 and 28 (Kramer and Ahlstrom 1968; Hewitt 1980).

shore only 260 km, but a 1-million ton spawning biomass would extend offshore 360 km.

Spawning Season

Some anchovy spawning takes place at every time of the year, but winter and spring are times of the most active spawning.

THE SAMPLING PROCESS

Catchability and Vulnerability of Adults

Epipelagic schooling fish are difficult to sample quantitatively. At times the sample trawls have been placed based on sonar mapping the previous day. At other times the samples have been taken where newly spawned eggs were detected by preliminary examination of plankton samples. Uniform samples of all tows conducted for the purpose of estimating egg production represent the 7 to 40-m depth range. The upper limit is set by the minimum depth at which a mid-water trawl can be fished.

One aspect of the variation in catchability can be illustrated by examination of the variation in the sex ratio. If one considers the data on 362 observations for which sex ratio was determined between 1977 and 1982, there is an interesting distribution of sex ratios and standard deviations of sex ratio by time of day (Table 2; Hunter and Macewicz 1980). For comparison, the overall mean of sex ratios is 0.497 with a standard deviation of 0.233 and a standard error of the mean ratio of 0.012.

The dispersion of values, about 50% female, is wider than one would expect of a binomial sampling error distribution with 10-30 specimens, thus we believe that the phenomenon reflects actual biological features of the anchovy schooling and behavior pattern. In further support of this idea, the distribution about 50% is skewed to the side of underestimation of females and the bias arises from a peak time 2200-2359 which coincides with the maximum spawning activity as seen from the surveys of stage I and II eggs (see Moser and Ahlstrom 1985). The strength and prevalence of this spawning behavior are demonstrated by the fact that a 5% overall bias in sex ratio may be caused by only 10-15% of females and their attendant males.

We postulate that during the spawning act more males than gravid females are present, and this leads to temporal and spatial heterogeneity; also, the collection of samples with a trawl from a volume which is 15 m thick, 15 m wide, and 2,000 m long has considerable chance of mixing these proportions by transecting spawning and nonspawning clusters. Thus it is that the modal catch category, 43 of 362 samples, is at 50% female. The binomial sampling theorem for fish sample sizes of 10-30 fish would indicate that about 6% of the samples would yield <30% female and also 6% would yield >70% female. The actual observations are given in Table 3.

For the use made of the adult data at present, the sex ratio bias (see Hunter et al. 1985) is not thought to be of any great importance: the ratio of 1-d postovulatory gonads is used for the inverse of the daily spawning fraction, and the sex ratio is determined to be 50% because there is no weight differentiation by sex and the numeric ratio is likely to be 50% as well. In another section (Alheit 1985) we shall see that the Peruvian adult sampling system with purse seiner exhibits no bias, and we assume that the explanation lies with the evasion of the trawl or the depth distribution of the sexes. In general, the commercial fishery exhibits a sex ratio of the order of 60% female, and the sea survey has a ratio of 50% female (Mais 1974).

The inference that changes in catchability and vulnerability of the adults are controlled somewhat by the spawning act is strongly supported by the coincidence of spawning detected in the sea. Statistics are available from 3,936 observations between 1980 and 1983 of which 1,666 had 1 or more anchovy eggs. Prior to the first cellular division (Stage I eggs, see Moser and Ahlstrom 1985) there are small numbers of observations and these reflect the temporal distribution of spawning in much the same way as the adult sex ratio variance (Table 4).

Table 2.—Percent females in trawl catches of northern anchovy at different times of day.

Time of day	No. of samples	% females	SD (%)
1800-1959	77	52.6	18.9
2000-2159	88	47.0	27.9
2200-2359	80	45.3	26.0
0000-0159	64	50.5	22.2
0200-0359	24	50.4	17.7
0400-0559	21	57.1	11.1
0600+	8	65.6	14.7

Table 3.—Actual and expected catches of females of northern anchovy at different times of day during spawning.

Mean time (h)	No. of samples	No. of samples expected at <30% and >70%	No. samples observed <30% female	No. samples observed >70% female
1900	77	5	6	13
2100	88	5	26	19
2300	80	5	22	12
0100	64	4	9	11
0300	24	1	1	3
0500	21	1	0	3
0600+	8	1	0	3

Table 4.—Percent positive egg captures and mean number of northern anchovy per tow by time of day.

Time	Observations	Positive samples	% positive	Mean no.
1800-1959	142	5	3.5	0.120
2000-2159	135	26	19.3	0.919
2200-2359	147	48	32.7	3.265
0000-0159	152	33	21.7	3.303
0200-0359	151	22	14.6	0.464
0400-0559	132	8	6.1	0.462
0600-0759	137	6	4.4	0.153
0800-0959	129	1	0.8	0.008
1000-1159	129	5	3.9	0.039
1200-1359	133	2	1.5	0.023
1400-1559	128	2	1.6	0.016
1600-1759	151	1	0.7	0.007

Sampling Requirements for Egg Survey

The egg survey supplies two values to the spawning biomass estimate: The size of the spawning area and the daily production of eggs per unit area. When establishing standards for the survey of egg production, it is necessary to consider the inclusion of the entire spawning area, the representativeness of the samples within the area, and the sufficiency of the number of observations for the required precision.

There is an obvious bias associated with egg production outside the surveyed area. The product of the survey area and the egg production/unit area is unbiased even if the survey is much larger than the spawning area. That is, as the surveyed area outside the spawning area increases there is a corresponding decrease in the mean number of eggs produced/unit of survey area. However, a secondary objective of the Southwest Fisheries Center Egg Production Method is to determine the error distribution of each estimated parameter. The Central Limit Theorem is not valid in reduction of standard-error-of-the-mean value with increasing number of observations if those values are spatially coherent. If there was a broad contiguous and continuous area of observations with no eggs in the observations, the standard error would be correspondingly biased as an underestimate.

It is not necessary to know the nature of the underlying distribution of observations to use the Central Limit Theorem to estimate the error distribution of the mean. However, it may be useful to consider some probability-generating distributions in order to forecast what an adequate number of observations would be, given the sample variance and the objectives of the survey. As an example of the differences one might encounter, consider: If the eggs were distributed such that the observations produced a "Normal" distribution, then with each sample one would obtain an independent estimate of the population mean and the population variance; if the eggs were distributed such that the observations produced a "Poisson" distribution, then the estimated mean of the population would simultaneously produce an estimate of the variance of the population (equal to the mean); if the eggs were distributed with areas of high density and areas with no eggs at all such that the presence of one egg would predict the presence of other eggs in the sample so that a "Negative Binomial" distribution of observations obtains, one would need many independent observations to obtain a mean number of eggs/unit area with a normal distribution of standard error of the mean. Thus the expense of effort of estimation-per-unit precision is much lower with "Normal" and "Poisson" distributions than it would be with an underlying "Negative Binomial" distribution. To diagnose this, one needs to compare the variance and mean of several estimates. If the variance is independent of the mean, the distribution is "Normal"; if the variance is a power function of the mean, the distribution is probably "Negative Binomial," although there are other possibilities. For a first approximation, the "Negative Binomial" appears to be a useful working model for design of the egg production survey to determine the mean and variance of the egg production parameters.

The establishment of the "Negative Binomial" model of underlying distribution of observations does not support speculation as to the scale or origin of the patchiness, and thus no single set of samples can be used to interpret pattern.

For the purpose of determining sampling requirements for an egg survey, it is necessary only to show that the number of samples is adequate to describe the mean and standard error of the production of eggs per unit area. A second level of analysis and sampling is necessary to jointly describe the scale and intensity of the patchiness and so allow speculation and research as to the processes which underlie the observed pattern. Knowledge of scale of the "patches" and intervening "spaces" and their shapes is necessary for further work.

We discuss the description of pattern below merely to emphasize what we would need to know to interpret processes such as:

1. The interannual variability in patch scale possibly caused by differences in fecundity attributable to such parameters as batch size, interval between spawnings, and age composition of spawners;
2. The geographic variability in the scale and intensity of predation;
3. The interaction of patch scales and numbers of anchovy eggs with patch scales and numbers of their principal predators, including adults within the school, adults and juveniles in other schools of the same species, and other noncannibalistic predators on anchovy eggs;
4. The relevance of interaction between the sampler size and the anchovy egg patch scale in order to transfer these sample designs to other anchovy populations or to other species; and
5. The comparison of vertical samples to older oblique samples, or the effects of taking samples of different size and shape on survey efficiency.

One reason that the standard sampling tactic (the vertical egg tow on a systematic grid) is not fully effective for advanced description of pattern is that observations are taken at one size scale and separated by a fixed distance. If we take the characteristics of the negative binomial—the mean, the coefficient of patchiness, "k", and the percent of the area, $P(0)$, with no eggs—only the mean is a characteristic of the sampled population; k and $P(0)$ result from interactions between the scale of the sampler and the scale of the patches of eggs. For example, if the vertical sampler mouth opening were progressively increased from 0.05 m^2 to 10 m^2, we would expect the probability of "zero" observations to decrease. The decrease would depend on what fraction of the interpatch spaces were between 25 cm (the diameter of the smaller sampler) and 357 cm (the diameter of the larger sampler). For k the equation for the population is:

$$k_i = \frac{\mu_i^2}{\sigma_i^2 - \mu_i}$$

where k_i is the scale-dependent coefficient of patchiness, μ_i is the population mean of all possible areas for sample size "i", and σ_i is the population variance for that set. If the true scale of anchovy egg pattern were approximately the same as the larger sampler, the sample variance would increase under the influence of larger observations and there would be a concomitant diminution of the variance from the effect of the larger sampler lying across more patch boundaries and thus integrating patch and space densities. If the true scale of the anchovy egg patches were smaller than the larger sampler, the variance would decrease because all observations would integrate some space densities. If the true scale of the anchovy egg patches were greatly larger than either sampler, the derived k's would tend to be indistinguishable.

Lastly, if the true scale of the anchovy egg pattern is larger than the spatial interval between stations, the variance will be less because adjacent observations will be more similar than observations chosen at random.

There is evidence that several scales of pattern exist which probably originate from different processes. The smallest scale is imposed by the process of fertilization and is at the scale of a single

female or a small group of females. The next scale dimension is imposed by the general schooling habit. The largest scale within the subpopulation is called the "school group" and it may contain several thousand schools of juveniles and adults in varying degrees of spawning condition. For the purposes of this discussion we can label these scales, meters, hundreds of meters, and thousands of meters: the biogeographic boundaries of the entire interbreeding subpopulation (central subpopulation of the northern anchovy) is 400 km cross-shore and 1,000 km along the coast.

The larger scales are of practical concern for the egg production method using the Central Limit Theorem; namely, the effect of multiple observations forming a normal distribution of the standard error of the mean is diminished by coherence among adjacent observations. Preliminary analyses of replicate egg samples (Smith and Hewitt 1985) indicate that cross-shore observation transects with observations separated by only 500-1,000 m would be coherent, while observations in excess of 5,000 m apart are independent of the major persistent source of patchiness at the "school" scale, 100-1,000 m. School groups have not yet been positively identified by the egg production surveys, but they are obvious from aerial and wide-ranging sonar mapping surveys (Fiedler 1978; Smith 1978).

To summarize, sample design decisions for the Southwest Fisheries Center Egg Production Method of biomass assessment have been based on prior knowledge, e.g., 1) the anchovy's spawning season, 2) the spawning area, 3) size of the school groups, and 4) size of the schools.

The volume of one observation is 3.5 m^3 or 1/20 m^2 between 70 m and the surface. The distance between observations is 4 nmi in the cross-shore direction and 10 or 20 nmi in the alongshore plane. For the central subpopulation of the northern anchovy, the recommended sampling area is 200 mi cross-shore and 600 mi alongshore.

Random vs. Centric Systematic Area Sampling

The sample design for the egg production method does not use random sampling: the lines are fixed in cross-shore positions which conform to historical surveys of biological and oceanographic features which can be measured only with straight sections at or near right-angles to the coast. The danger with fixed transects is that characteristics, such as proximity to a canyon or upwelling site, the passage close to an island, or the aversion to stations in shipping lanes, all adversely affect the necessary assumption that all objects to be sampled have had an equal opportunity to appear in a sample. Another problem is the regular spacing of systematic samples: if the alongshore or cross-shore spacing coincides with any periodic element in the distribution of the organisms being sampled, then the possibility exists for bias through oversampling some phases of the spatial periodism. For example, if the number of organisms varied like a sine wave, it is possible that regularly placed samples will hit the peaks or troughs in the distribution, thereby over- or underestimating the population. In a strict sense, statistical limits cannot be established with sets of systematic samples: there is some evidence (Milne 1959) that for periodic differences to be important, they would have to be obvious. Milne (1959) stated ". . .with proper caution, one will not go very far wrong, if wrong at all, in treating the centric-systematic-area sample as if it were random."

INITIATING AN EGG PRODUCTION PROCEDURE

The Southwest Fisheries Center Egg Production Method was created using new principles of sampling and mortality analysis of anchovy eggs in the sea and residual gonadal tissues indicating recent ovulation. There existed a wealth of data on the geographic distribution of adults (Mais 1974) and the geographic and temporal distribution of anchovy spawn (Kramer and Ahlstrom 1968). It should not be necessary to repeat the CalCOFI effort at each site and for each species to develop egg production procedures for other species in other geographic areas. It is the purpose of this section to describe minimal initial steps for the egg production method.

Initiation of Field Sampling

Observations of larger larvae (5-10 mm) from high-volume oblique tows (ca. 1,000 m^3) would be superior to the CalVET (3.5 m^3) tows for establishing the regional boundaries and season optimum for the egg production method for a given species. For widespread pelagic species exhibiting multiple spawning, interstation distances of 40-120 nmi should suffice (74-222 km) to delimit spatially the spawning area. Monthly, bimonthly, or seasonal surveys should be sufficient.

Laboratory and Field Studies of Spawning Behavior

While it is conceivable that temperature-dependent rates of egg development can be derived from the analysis of field samples and ancillary depth and temperature data, it is recommended that laboratory-controlled temperature experiments be used to establish the temperature-dependent rate model. In particular, the extremes of temperature may be difficult to observe adequately in the field in any given year.

In the field, even when there is no main time of day for spawning, it is possible to determine the number of eggs spawned per day by:

$$N_t = N_o \int_{-\infty}^{t} \exp[-Z(t-x)] f(x)\, dx$$

where N_t is the number of eggs in a particular stage and later stages at time t; N_0 is the total number entering the series; $\exp[-Z(t-x)]$ is the probability that an egg at time x is still alive at time t; Z is the constant age-specific death rate; and $f(x)$ is the frequency of the stage at time x (Manly 1974). The actual frequency function can be inserted in the equation. For example, a normal distribution of spawning time would yield the equation:

$$N_t = N_o \exp(-Zt) \int_{-\infty}^{t-\mu/\sigma} (2\pi)^{-1/2} \exp(-1/2x^2)\, dx$$

One must, of course, have more than four samples to solve this equation (Manly 1974).

When regional and seasonal criteria for spawning have been established, there must be an intensive field study to find the sample distribution in time and space which will be sufficiently precise for the purposes of egg production estimate of spawning biomass. The conditions which must be noted from the data set are (after Southwood 1978): 1) Distribution of the time of spawning, 2) variability of survival rates, 3) stage-dependent sampling efficien-

cy, 4) duration of stages, and 5) probability distribution of eggs as a function of time and age.

Working assumptions for the central population of the northern anchovy at lat. 33°N in March are:

1) Spawning occurs between 1800 and 0200, essentially normally distributed with a midpoint of 2200 hours.
2) The survival rate is assumed to be constant between 0200 of the first day after spawning until hatching begins (normally 60 h).
3) All stages are sampled with equal efficiency, but the period when spawning is actually occurring or after hatching begins is excluded from survival stimulation.
4) Duration of egg stages is proportional to a single temperature (usually the surface temperature).
5) The probability distribution of eggs/unit surface area is completely characterized by the two parameters of the negative binomial distribution, the arithmetic mean and the dispersion parameter k, a function of the population mean and variance. These parameters are age-specific, the mean decreasing with age and k increasing with age.

The last assumption must be considered for the regression method of estimating the precision of the slope and intercept estimates, as there are, as yet, no formal solutions for regression equations under these conditions.

Staging Eggs in Other Species or in Tropical or Arctic Habitats

The anatomical description of the 11 stages of eggs is given for anchovy in Moser and Ahlstrom (1985). Also, an experimental definition of the mid-age of each stage and the conversion of stage, time-of-tow, and temperature information are described by Lo (1985). We here describe some approaches to fishes whose spawning behavior and definition of stages are dissimilar to the anchovy.

The possible number of discrete stages assigned to the continuum of embryonic development is ultimately determined by the presence of recognizable anatomical features. For example, although not used in this work, the 2-cell, 4-cell, and 8-cell stages of the embryonic development could be used to follow population features immediately after fertilization. For convenience the nominal hatching time of 60 h has been divided into 11 stages for a mean duration of about 6 h. If one were dealing with a tropical fish with a hatching time of 20 h, it would seem more reasonable to maintain the number of arbitrarily defined stages at 10 or so, rather than to reduce the number of stages to 3 of about 6-h length. Similarly in the Arctic situation, where hatching may take several weeks, one may need to pool several days' spawning to gain sufficient sample sizes to estimate mortality rate and egg production.

Were the stages equal in duration and spawned at an instant, one would easily see the progression of these stages with time. Since the period of spawning is approximately 1/4 d, and the duration of the stages is from 2 to 9 h, the system used here was originated for the sardine (Ahlstrom 1943) temperature-specific development rate and later applied to the anchovy (see below). Since these are both temperture-zone clupeoid fishes, the rationale for these stages is listed below for the purpose of using this technique for fishes in other latitudes or taxa.

While it has not been possible to obtain exact information on fertilization time or the exact timing of the transitions between stages, it is possible to combine quantitative laboratory and field data to make a best description of the major events in embryogenesis. This description can then be used to design definitive work in the laboratory or field as needed for future studies on these or other fish. We base all of the arbitrary descriptions on a starting time of 1800 (6 p.m.) for onset of spawning, with the midpoint of spawning at 2200 (10 p.m.), and finishing at 0200 (2 a.m.). Bolin (1936) observed that cell cleavage in the anchovy embryo occurred at approximately half-hour intervals, thus we assume that the interval between fertilization and first cleavage is about 0.5 h. Since the duration of the Stage II eggs from first cleavage until the onset of epiboly or cellular overgrowth of the yolk is about 7 h and the number of Stage II eggs collected is about seven times the number of Stage I eggs, we assume that the Stage I egg persists about 1 h or 0.5 h to be fertilized and 0.5 h more until the first cleavage.

Table 5 is from a summary of all field data taken in 1980-83, with 1,666 samples positive for some stage of anchovy egg. For each of the first five stages, the mean number of eggs per 0.05 m^2 is listed for each of 12 2-h periods, and next to each abundance is the cumulative percentage from the onset of the stage to the end of 24 h. For the cumulative percentage of each stage the point at which 5% of the eggs have appeared, 50% and 95% points on the cumulative curve are listed at the bottom of the table. The cumulative effects of temperature have little effect on the early stages. The sets included here are from all temperatures encountered in the surveys, mostly between 13° and 16.5°C in these years with an average temperature of 15.4°C.

In the laboratory experiment, it was not convenient to constrain the gravid anchovies, thus it is not known for certain when the first spawning begins under controlled conditions. Following spawning in the laboratory, the eggs were collected in the outflow in a passive net and were transferred into containers with temperatures controlled at approximately 13.5°, 15.1°, and 16.3°C. This means that the early stages concluded their development at a common temperature of about 15°C and were subsequently placed in controlled temperatures. Thus, for the tables which follow, the temperature is that at which most of Stage III and all of the ensuing stages were passed. The original data were somewhat more finely grouped, but low numbers of specimens in some categories and brevity made some lengthening of observation intervals desirable. To unify these numbers, the raw data were converted to numbers per hundred collected at each time interval, and then the numbers were summed for each stage and the cumulative sum at each reported interval was divided by the total to yield the tabled value of cumulative percent. The 0 in the table means that no specimens of that stage were spotted before that interval; the 1.000 means that no specimens in that stage were found after that interval. All Stage I eggs had developed before the eggs were caught at the outflow and most Stage II and some Stage III eggs were present before the systematic counting and staging began. The experiment was terminated before all larvae had reached the 3.5-mm length.

The actual times of day of the important events were injection of hormones in the afternoon of the preceding day and collection of the eggs in the morning. The first staging ensued at about 1300.

For any small set of anchovy samples, Stage I and Stage XI appear to be too short for any analytical purpose: each appears to be about an hour long. The other stages probably last between 5 and 13 h, and these durations diminish with increasing temperature and development. Thus for other species in lower and higher water temperature, the detection and use of a daily cycle of spawning should include redefined stages which are markedly shorter than one day at the beginning of development. The ensuing stages can be longer as is convenient for data gathering and storage.

Table 5.—Tabulation of field abundance of northern anchovy eggs as a function of time in hours after spawning; 00 refers to the mid-point of spawning.

	Stages									
	I		II		III		IV		V	
Age in hours	eggs/ 0.05m^2	cum. %	eggs/ 0.05 m^2	cum. %	eggs/ 0.05 m^2	cum. %	eggs/ 0.05 m^2	cum. %	eggs/ 0.05 m^2	cum. %
−04-02	0.120	0.014	0.113	0.002						
−02-00	0.919	0.118	1.511	0.026						
00-02	3.265	0.490	6.252	0.125						
02-04	3.303	0.866	11.612	0.308						
04-06	0.464	0.919	10.298	0.471						
06-08	0.462	0.972	8.932	0.613	0.197	0.004				
08-10	0.153	0.989	11.358	0.792	2.029	0.047				
10-12	0.008	0.990	7.426	0.910	4.767	0.149				
12-14	0.039	0.995	3.969	0.973	5.178	0.259	0.109	0.003		
14-16	0.023	0.997	1.023	0.989	7.474	0.419	0.316	0.013		
16-18	0.016	0.999	0.258	0.993	8.945	0.609	1.109	0.048		
18-20	0.007	1.000	0.450	1.000	8.762	0.796	2.378	0.121		
20-22					2.831	0.856	5.056	0.278	0.873	0.026
22-24					1.926	0.897	5.911	0.462	2.482	0.102
24-26					3.408	0.970	7.388	0.691	2.599	0.108
26-28					1.105	0.994	6.842	0.903	3.421	0.284
28-30					0.298	1.000	1.411	0.947	4.570	0.422
30-32							0.318	0.957	5.576	0.576
32-34							1.241	0.995	5.263	0.751
34-36							0.155	1.000	3.318	0.851
36-38									2.078	0.914
38-40									1.181	0.950
40-42									1.109	0.984
42-44									0.543	1.000
Age at which										
5% appeared	−1.5 h		0.5 h		10 h		18 h		22.5 h	
50% appeared	0.0		6.0		16		24		30	
95% appeared	4.5		13.5		25.5		31		40	

Abnormal Embryos

There appears to be a need for stages which can be recognized even with a distorted specimen. A large fraction of the anchovy eggs are decidedly abnormal and stage classification is quite difficult. We have sought to modify sample washing and fixation to diminish this problem, but the specimens are distorted when fresh and alive (Sandknop and Stevens[2]). Also these abnormal embryos do not seem to be associated with any particular stage or age of egg. Table 9 is a correlation matrix for the disintegrated eggs and the aged ones. The highest correlation is between disintegrated and total eggs, and even this shows no more than 7% of the number of disintegrated eggs can be predicted from the number of eggs in a sample.

Table 6.—Correlation matrix among categories of northern anchovy eggs.

Age of eggs	<8 h	1 d	2 d	3 d	4 d	DIS+[1]
1 d	0.101					
2 d	0.029	0.396				
3 d	−0.022	0.176	0.277			
4 d	−0.024	0.029	0.002	0.325		
DIS+	0.045	0.150	0.143	0.111	0.073	
Total eggs	0.388	0.786	0.737	0.465	0.130	0.258

[1]DIS = disintegrated eggs but with chorion intact.

[2]E. M. Sandknop, Biological Technician, and E. L. Stevens, Fishery Biologist, Southwest Fisheries Center, National Marine Fisheries Service, NOAA, P.O. Box 271, La Jolla, CA 92038, pers. commun. Dec. 1981.

The time in the field of the proportion of the spawning area covered by spawn (Fig. 2), the mean number of eggs per observation, the standard deviation, the standard error of the mean (Fig. 3), and the dispersion coefficient k of the negative binomial (Fig. 4) are consistent with the anchovy laboratory data and that for the Pacific sardine analyzed by Ahlstrom (1943).

SUGGESTIONS FOR FUTURE RESEARCH

Three characteristics of the anchovy make the assignment of ages possible: One is that the anchovy egg is demonstrably spawned during a limited period in each day; another factor is that stages have been assigned so that their duration is about one-third of a day so that ages can be unequivocally assigned if the temperature and time of tow is known; lastly, the total incubation time is about 3 d (see Table 7).

This leads one to the questions: How would one estimate egg production if the eggs were produced at all hours of the day? How would one proceed if the total incubation time were <1 d?—more than 10 d?

Research on these questions could proceed along these lines. For the case of the long incubation period, it is not necessary to follow the course of mortality through the entire incubation period. For example, if the incubation period were 14 d and the ages of eggs could readily be determined for only 3 d, the mortality and production rate from that period would suffice for an estimate of the production of spawn; the ensuing stages of eggs would not even have

to be counted or staged.

The rate of production could probably be estimated without knowing the time-of-day of spawning. For example, in Table 8 estimates of rate of production could be determined solely from empirical abundance information and incubation time. For the purpose of this exercise, I have assumed that the 11 stages are incubated in 66 h, or 6 h/stage. Deviations of abundance from the regression estimate of abundance are of two kinds: Sampling variability and duration differences from the even duration assumption. If sampling variability is ignored, then stage lengths longer or shorter than the average stage duration can be estimated.

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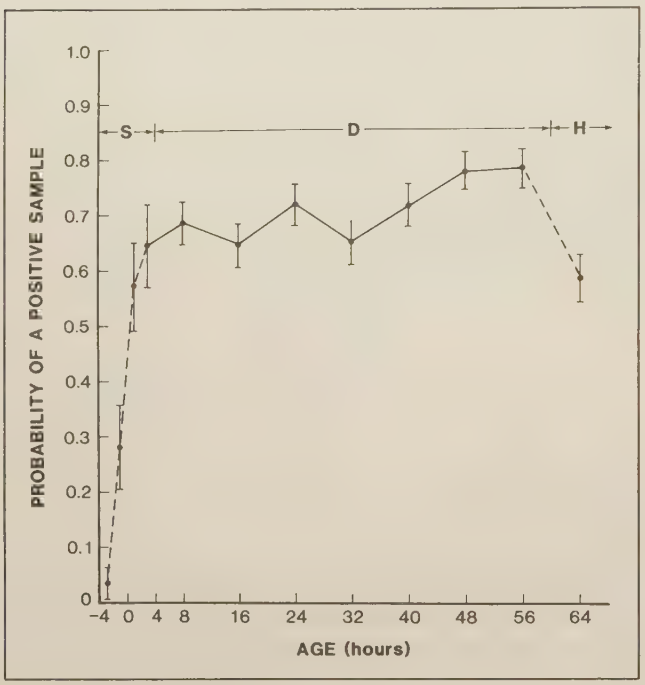

Figure 2.—Time course of proportional occurrence (number of observations with eggs of a given age divided by the number of observations with any age anchovy egg). Dashed lines under "S" indicate observations during the spawning period. The dashed line under "H" represents observations during the hatching period. The process assumed to control the solid line under "D" is dispersal.

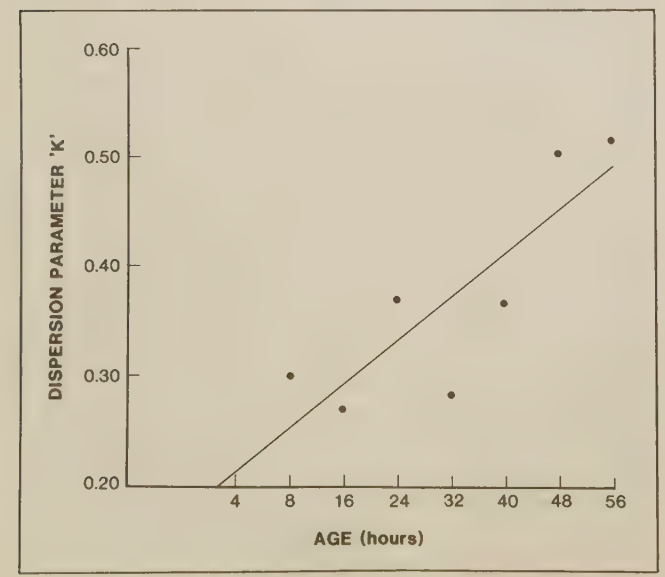

Figure 4.—Negative binomial distribution is represented by two parameters: the arithmetic mean (see Figure 3) and "k", the "dispersion parameter." The points represent maximum-likelihood fits to sample data in seven 8-h intervals which contain samples unbiased by spawning or hatching. The line represents the linear least-squares fit to these points.

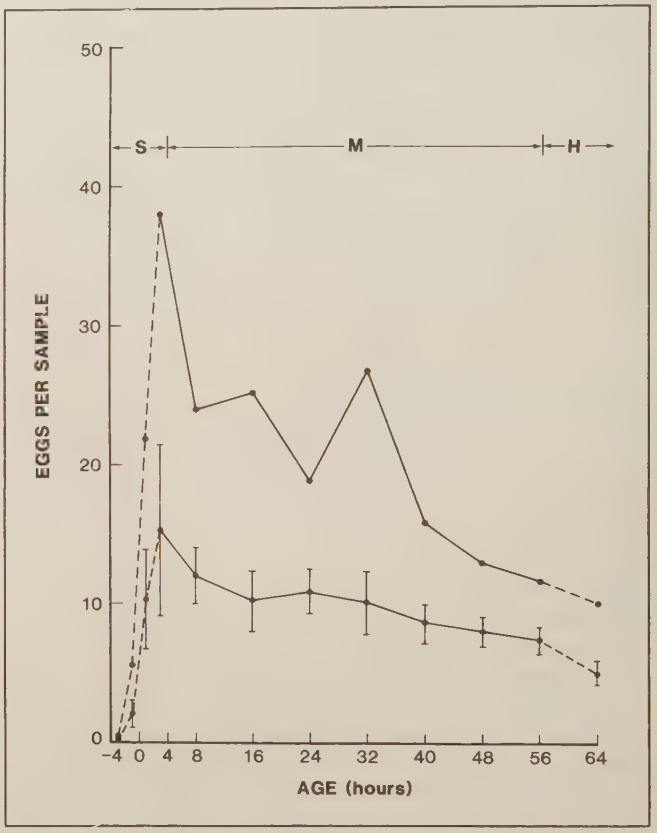

Figure 3.—Time course of the primary statistical parameters, the arithmetic mean (below), the standard deviation of the observations (above), and the standard error of the mean (cross-bars on the arithmetic mean line). Within ages the standard deviation is believed to be a function of the mean, and this function of the mean changes with time owing to dispersal (see Figure 4). Standard error of the mean bars are ±2 (or approximately the 95% limits). For this illustration the number of observations is 1,666 taken between 1980 and 1983 as part of the egg production method estimate of anchovy spawning biomass. The dashed lines under "S" and "H" represent spawning and hatching as in Figure 2. The principal process controlling the slopes of the standard deviation and mean under "M" is mortality. The convergence of the standard deviation and mean lines is caused by dispersal of eggs.

Table 7.—Cumulative incidence of laboratory-spawned and reared northern anchovy eggs and larvae.

Age	Stage of development							Larvae	
(h)	IV	V	VI	VII	VIII	IX	X	2.5 mm	3.0 mm
16	0				at 13.5°C				
24	0.226	0							
32	0.812	0.204	0						
40	0.995	0.723	0.128	0	0				
48	1.000	0.985	0.759	0.265	0.032	0			
56		1.000	0.992	0.907	0.552	0.032	0	0	
64			1.000	1.000	1.000	0.684	0.174	0.100	0
72						0.947	0.802	0.292	0.004
80						1.000	1.000	0.724	0.014
88								0.880	0.271
96								0.975	0.554
104								1.000	0.763
112									0.843
120									0.888
128									0.977
136									1.000
16	0.028	0			at 15.1°C				
24	0.591	0.010	0						
32	0.963	0.505	0.075						
40	1.000	0.965	0.459	0	0				
48		1.000	0.974	0.458	0.016	0			
56			1.000	0.981	0.874	0.273	0	0	
64				1.000	0.976	0.929	0.500	0.145	0
72					1.000	1.000	1.000	0.436	0.078
80								1.000	0.230
88									0.527
96									0.810
104									0.868
112									1.000
16	0.025	0			at 16.3°C				
24	0.766	0.069	0						
32	0.944	0.881	0.231	0					
40	1.000	1.000	0.923	0.570	0.037	0			
48			1.000	0.974	0.880	0.210	0	0	
56				1.000	1.000	0.746	0.387	0.250	0.004
64						1.000	1.000	0.625	0.046
72								0.837	0.367
80								1.000	0.685
88									0.930
96									1.000

Table 8.—Empirically derived stage durations of northern anchovy eggs.

Stage	$\bar{X}$	2SE	4X	T	RE	D	CUM	F	L	d
I	0.77	0.28	3.08	3	13.78	1.34	0- 1.34	0.67	—	
II	5.34	0.80	21.36	9	12.85	9.98	1.34-11.32	6.33	6.42	−0.09
III	3.86	0.76	15.44	15	11.98	7.73	11.32-19.05	15.19	13.55	1.64
IV	2.79	0.48	11.16	21	11.17	6.00	19.05-25.05	22.05	21.12	0.93
V	2.75	0.62	11.00	27	10.41	6.34	25.05-31.39	28.22	30.70	−2.48
VI	3.06	0.44	12.24	33	9.71	7.56	31.39-38.95	35.17	39.24	−4.07
VII	2.28	0.36	9.12	39	9.05	6.04	38.95-44.99	41.97	47.13	−5.16
VIII	1.65	0.22	6.60	45	8.44	4.69	44.99-49.68	47.34	52.07	−4.73
IX	2.45	0.14	9.80	51	7.87	7.47	49.68-57.15	53.42	56.28	−2.86
X	1.86	0.10	7.44	57	7.34	5.92	57.15-63.07	60.11	62.63	−2.52
XI	0.44	0.074	1.76	63	6.84	1.54	63.07-64.61	63.84	65.65	−1.81

$\bar{X}$ = mean number of eggs by stage (n = 1,666) per observation
SE = standard error of number of eggs by stage.
4X = daily production by stage if duration is 6 h per stage.
T = midtime of stage if duration is 6 h per stage.
RE = regression estimate of abundance (first approximation).
D = duration of stage in hours if regression error is ignored.
CUM = cumulated age of stages in hours.
F = midtime of each stage estimated from field data.
L = midtime of each stage estimated from lab experiment.
d = difference in hours.

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The CalCOFI Vertical Egg Tow (CalVET) Net

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ABSTRACT

The vertical egg tow (CalVET) net was devised by CalCOFI (California Cooperative Oceanic Fisheries Investigations) to estimate egg production in the central subpopulation of northern anchovy and similar fishes. This paper describes the sampling characteristics of the net and its interaction with physical and distributional features of the anchovy egg. Problems discussed include the horizontal patchiness of the eggs and intensity of their distribution, volume and depth distribution of the water filtered, egg retention, and towing characteristics of the net.

The mouth area of the CalVET net is 0.05 m^2; the tow is vertical to minimize the volume of water filtered per unit depth; the mesh size of 0.150 mm is selected for total retention of the anchovy eggs under all likely conditions. The mesh area of the net is three times the mouth area in the conical portion and five times the mouth area in the cylinder. The conical mesh is the minimum size necessary for highly efficient filtration, while the cylindrical portion reduces the probability of the net clogging during a single tow. A flowmeter detects sequential clogging of the net during a series of tows. The net is lowered and raised rapidly to diminish the effects of ship drift and undersea currents which impose uneven trajectories on the net. The net is probably not capable of sampling active larvae 5 mm or longer, owing to the small mouth size and the disturbance to the net's path from the towing wire.

Design and working drawings of the frame and net are included.

HORIZONTAL PATCHINESS

We assume that eggs are released in the open sea by the gravid females of a northern anchovy school in close proximity to one another where they are fertilized by males. Previous samples of anchovy and sardine eggs indicated that most are found at densities of 1,000 to 5,000 eggs/m^2 when spawned, although only a small proportion of the samples actually contained eggs at these densities (Smith 1973; Smith and Richardson 1977:84). There is also evidence that most eggs are found in the upper 70 m of the water column (Ahlstrom 1959; Pommeranz and Moser 1983).

To shorten the sorting time for samples, we used a small net, 0.05 m^2 in cross section. To reduce the time spent sorting out the anchovy eggs from plankton, we used a vertical tow through the upper 70 m rather than a normal oblique tow, since a vertical tow captures far less extraneous plankton. Sample simulations on existing data for unstaged and staged anchovy eggs indicated that adequate sample precision could be obtained with 500 to 1,000 positive egg samples.

Previous studies by sonar, oblique plankton tow samples, and observations of spawning in the laboratory indicated three contributors to horizontal patchiness of anchovy eggs in the sea: 1) The act of fertilization; 2) the schooling habit; and 3) the propensity of schools to be contagiously distributed. The expected scale of horizontal patchiness from the act of fertilization is of the order of meters to tens of meters. The expected scale of horizontal patchiness from the schooling habit ranges from hundreds to thousands of meters. The expected scale of patchiness from the school group scale is of the order of kilometers to tens of kilometers. A study using eight replicate tows per station identified the school scale as the primary source of variance. Samples replicated at the same station indicated that only during the first half day after spawning was the fertilization swarm a significant source of variance relative to the school-size scale. To achieve statistical independence among adjacent samples, we decided to space the samples at least 3 km apart. The most usual spacing between samples has been 6 km in the offshore-onshore direction and about 16 or 32 km in the alongshore direction.

DEPTH DISTRIBUTION OF WATER FILTERED

The design objective of the CalVET tow was to filter a uniform and measured volume of water for each increment of depth from 70 m to the surface. The chief barriers to this goal are 1) ship drift and undersea currents, 2) clogging of the net, and 3) cyclic vertical motions of the towing wire caused by the heave and roll of the ship (Hewitt 1983).

The primary effect of ship drift and undersea currents is to distort the distribution of water filtered/unit surface area to a given depth. The ideal tow as designated here is to sample about 50 L water/m of depth to a depth of 70 m, or to near-bottom in shallow waters. First of all, ship drift and undersea currents diminish the depth attained with the fixed length of towing wire. For example, if the wire has strayed from the vertical by 30°, 70 m of wire will achieve only 61 m depth. Similarly 5° stray will affect the depth attained by less than 1 m. Secondly, ship drift and undersea currents change the angle of stray with time. For example, the net when vertical will filter 50 L water/m of depth, and at 30° the net will filter 58 L water/m of depth. Our usual experience is that the oversampling occurs in the upper layers relative to the deeper layers. In the recommended sample series all tows are repeated when the angle of stray has exceeded 30°.

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The most important specification in this category is that the net must emerge from the water at right angles to the surface. It is known from horizontal samples at the surface that great concentrations of eggs may exist there, thus to drag the net along the surface toward the ship is to risk vast oversampling of the eggs.

The chief error caused by clogging of the meshes of the net is the relative undersampling of the shallow vs. deep water in an ascending vertical tow. It is conceivable that a net which is filtering 50 L/m of depth might clog in the upper layers and filter 40 or fewer liters per m of depth (Smith et al. 1968). The viscous resistance of the filtering surface requires that about three times as much mesh aperture as mouth aperture is required to filter water efficiently. For this reason the CalVET net is fitted with a truncated conical portion which contains three times as much mesh aperture area as the opening of the net. Since the mesh chosen is about 46% open area, the 0.15 m^2 aperture area of the conical section is achieved with 0.33 m^2 of mesh. In addition, to diminish the danger of the net changing filtration efficiency during the tow due to clogging, the net is fitted with a mesh cylinder with five times the mesh aperture area compared with the mouth opening of the net. This should be ample for a tow this length and with this mesh size (0.333 mm; see below) in coastal waters off California.

Filtering efficiencies of 97% are possible with simple net apertures. Efficiencies of 100% and more are possible with inverted conical net entrances like the original Hensen net. The complicated Hensen technique was not chosen because the filtration efficiency becomes a function of net speed, and the placement of the flowmeter in the flow section to obtain a representative sample of water filtered is poorly understood. Also, reverse cone structures increase the lateral drag of the net and worsen the consequences of ship drift and undersea currents. The design of the CalVET net makes clogging unlikely within a tow; thus the use of a flowmeter is not required to verify the constant filtration of water per unit depth. However, nylon nets are subject to sequential clogging in a series of tows, and flowmeters are recommended to ascertain and correct this problem. Deviations of flowmeter revolutions of 10% or more should be cause for exchange or careful washing of the net. For example, a calibration factor of 0.273 m/revolution of the flowmeter would predict that 256 revolutions be characteristic of a suitable tow from 70 m to the surface. Flow readings of 231 revolutions for the full tow, or 3.3 revolutions/m of wire out in shallow tows, would indicate that a sequence of clogging between tows may have begun.

Another retention problem with vertical net tows relative to horizontal and oblique tows is the direct connection of the net to the ship. The roll and heave of the ship could at times impart velocities to the net less than or in excess of design expectations. Several consequences are possible between motion of the net and the ship's roll and heave. The most serious problem is noted with simultaneous roll and heave downward when the net containing eggs approaches the surface (Hewitt 1983). Should the downward velocity exceed the recovery rate of the net, the net would collapse from below, there would be reverse filtration of water through the mesh apertures, and the eggs would be expelled from the mouth of the net. Probably the next most important danger is that when the net containing eggs approaches the surface, the simultaneous roll and heave upward will add the speed of the ship's motion to the already high speed of the net recovery wire. In this case, the eggs lying on the mesh might be ruptured or extruded through the mesh apertures. Quantitatively less important, perhaps, is the likelihood that a net at depth might be propelled upward fast enough to filter water and turn the flowmeter, thus oversampling the volume at that depth. The flowmeter is normally jammed against the mesh on the downward path. To diminish these effects, the net is lowered at 1-1.2 m/s and recovered at the same speed. In this way we achieve a depth of 70 m in 1 min and recover at the same speed. The net is stopped for 10 s at the bottom. Tows which vary substantially from this regimen should be repeated.

RETENTION OF ANCHOVY EGGS

The main considerations in retention of anchovy eggs by the CalVET net are the relative size of the egg, the mesh aperture, and the filtration velocity. The minimum dimension of the northern anchovy's egg is about 0.65-0.85 mm in February, and 0.55-0.80 mm in August (Smith and Richardson 1977:61). Such an object should be fully retained by mesh sizes <0.4 mm according to the "diagonal rule." The diagonal rule states that the minimum dimension of the object to be sampled should be greater than the diagonal of the mesh aperture. Absolute retention is affected also by the filtration velocity. For example, the CalVET net proceeds through the water at a nominal velocity of 70 m/min. The active filtering surface is about three times the net mouth area, thus the filtration velocity is 1.2 m/s divided by 3, or 0.4 m/s. This is a high filtration velocity relative to the normal oblique tows. Thus the net mesh size for the CalVET was selected to be 0.150 mm. The main reason for the high net velocity is to diminish the time for errors of ship drift and undersea current to accumulate.

DESIGN AND WORKING DRAWINGS OF THE CalVET NET

The CalVET frame and net are much smaller than traditional ichthyoplankton nets: 0.25 m diameter mouth opening (0.05 m^2 mouth area) and the total length of the net is <1.5 m. The net is lowered to a depth of 70 m and retrieved vertically at 70 m/min; strict controls govern the field procedures, and a tow is rejected if the wire strays too far from the vertical or if the retrieval rate is too fast or too slow. The results from field testing the gear in 1979 indicated a very high degree of fidelity among eight replicates at a single station; the test was conducted over 70 stations and within a 200-fold range in egg abundance.

Figures 1-5 describe the bongo-type (PAIROVET) version of the CalVET sampler. The frame was designed to facilitate comparison of nets constructed of various materials and to provide replicate observations when using similar nets; it has become one of the standard samplers used by the Southwest Fisheries Center. The frame is constructed of 6061-T6 aluminum with stainless steel fittings. The nets are nylon mesh attached to the frame with adjustable stainless steel strapping. The cod end is removable and attached to the rest of the net by means of a plastic collar. The net and cod end are sewn inside a "sleeve" constructed of 2-mm mesh vinyl-coated polyester (not shown) to protect them from abrasion.

Nets of several mesh sizes have been used by the Southwest Fisheries Center. All have been constructed using the dimensions shown in the figure; total area of net material is 0.95 m^2. With the exception of the 0.035-mm mesh, all of the net materials have approximately the same porosity, i.e., the portion of the net area which is open. Smith et al. (1968) suggested that adequate filtering efficiency can be maintained in coastal waters if the ratio of aperture area to mouth area is at least 3.4 for a net of the proportions described. Thus, these nets may be expected to perform well with the exception of the very fine (0.035 mm) mesh net which may clog in turbid coastal waters (Table 1).

Table 1.—Ratio of aperture area to mouth area for various net mesh sizes. Nets with mesh <0.333 mm are protected by a 2-mm mesh nylon sheath. See text.

Mesh (mm)	Total area (m^2)	Porosity	Aperture area (m^2)	Aperture area/ mouth area
0.035	0.95	0.16	0.15	3.04
0.075	0.95	0.45	0.43	8.55
0.150	0.95	0.51	0.48	9.69
0.250	0.95	0.49	0.47	9.31
0.333	0.95	0.46	0.44	8.74
0.505	0.95	0.49	0.47	9.37

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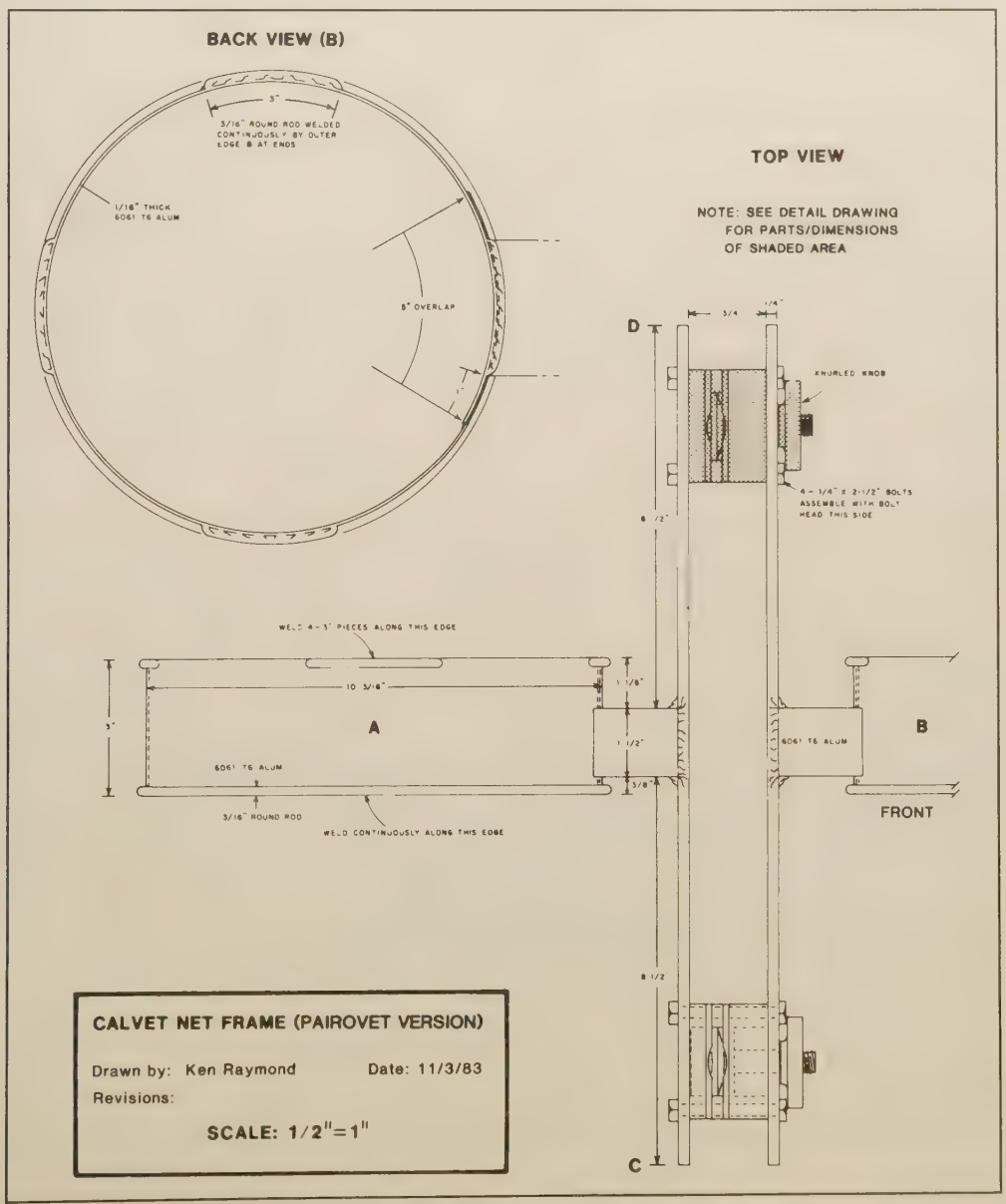

Figure 1.—Back and top view of PAIROVET version of CalVET net frame.

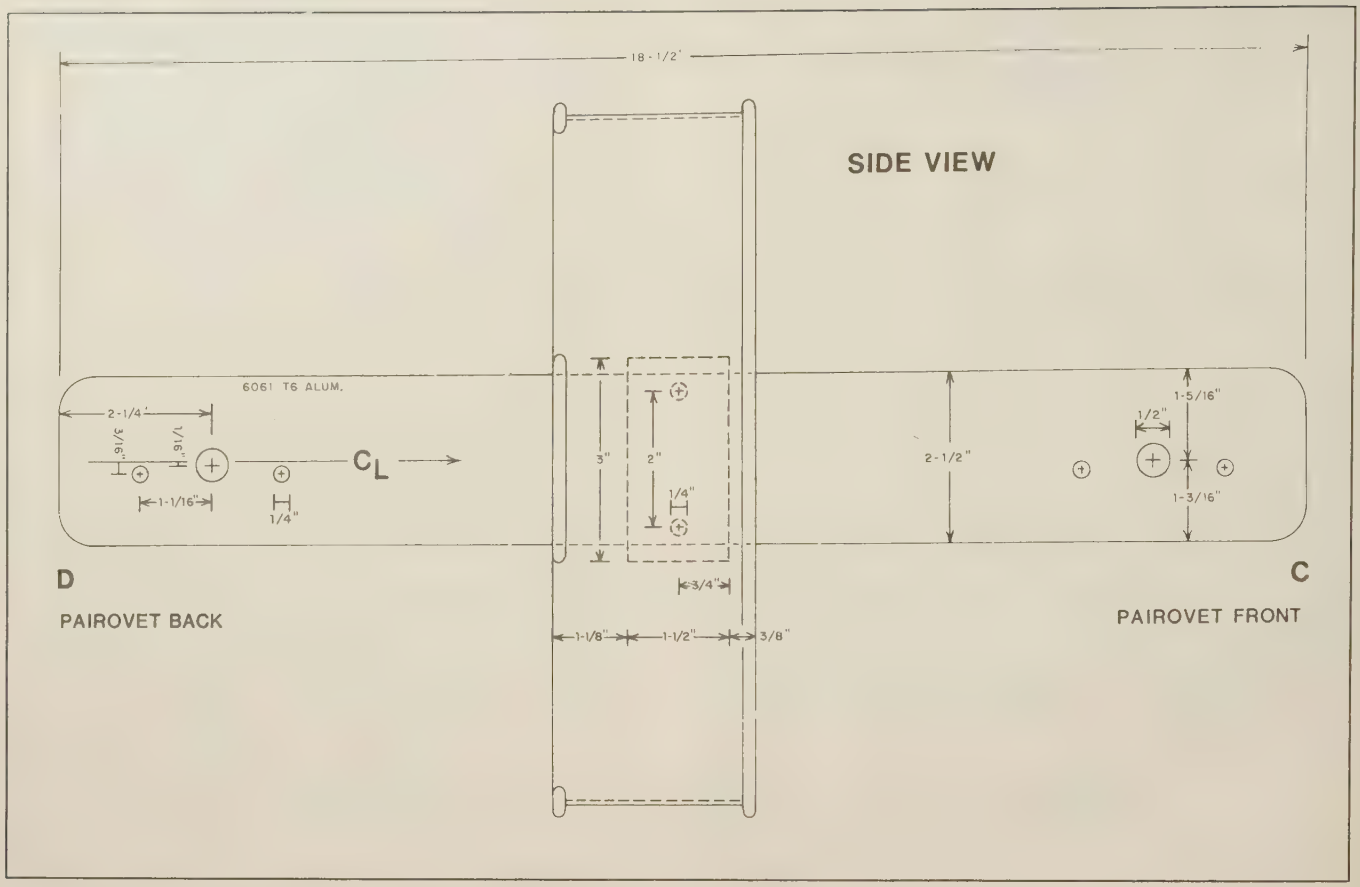

Figure 2.—Side view of PAIROVET net frame.

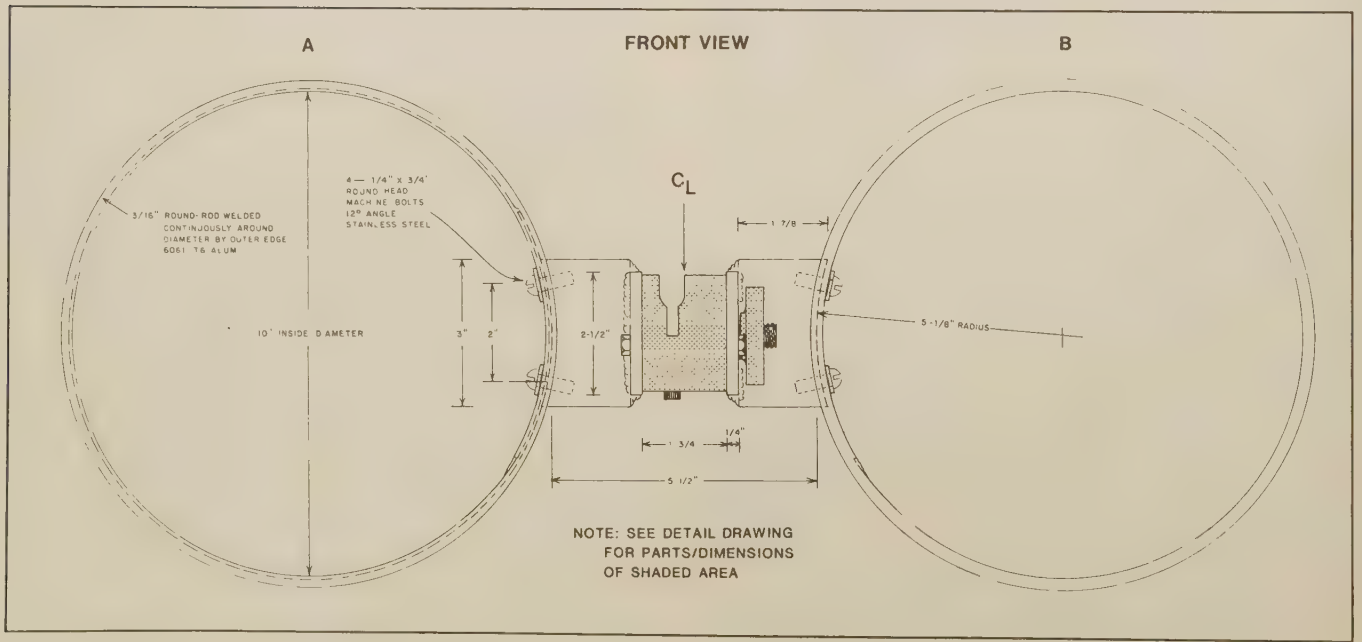

Figure 3.—Front view of PAIROVET net frame.

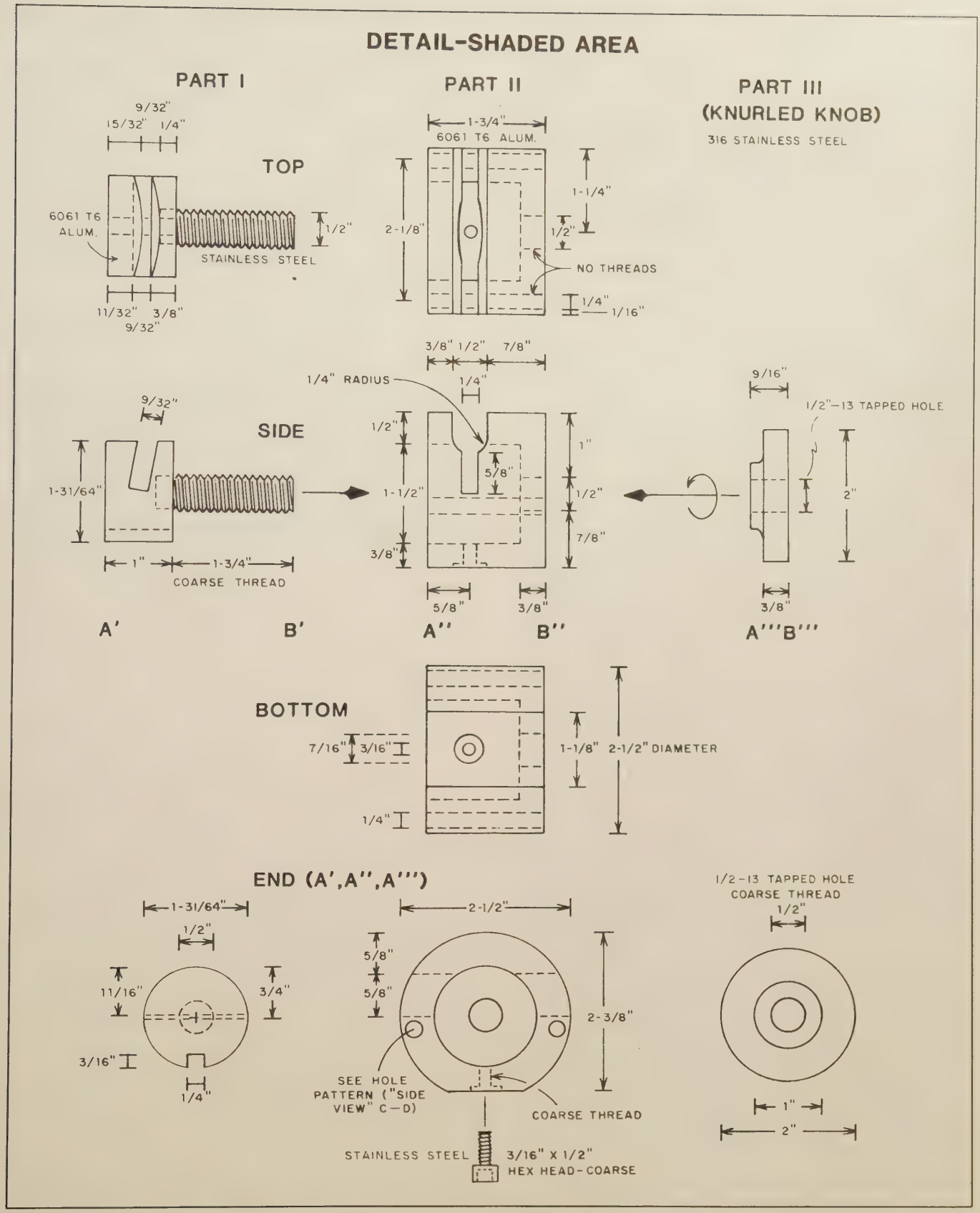

Figure 4.—Detailed view of wire attachment for PAIROVET net frame. "Shaded area" refers to Figure 3.

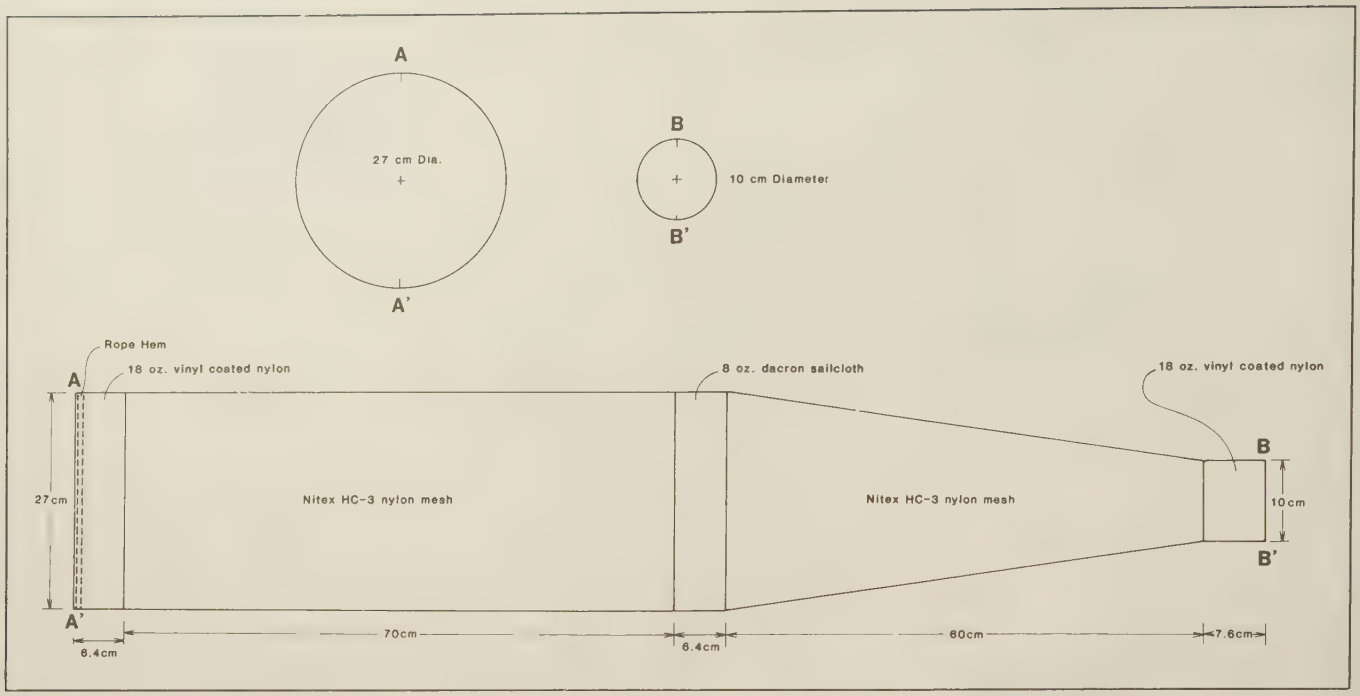

Figure 5.—CalVET net dimensions. For nets with mesh sizes <0.333 mm, a protective sheath of 2-mm mesh nylon is used. See Table 1.

Procedures for Sorting, Staging, and Ageing Eggs

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ABSTRACT

Estimation of the daily production of eggs spawned is based on data for the number of eggs sampled during the plankton survey. This requires that the age of individual eggs be determined. To accomplish this, eggs of the surveyed species must be sorted out of the plankton samples, staged according to their state of embryonic development, and aged. The initial step in processing the plankton samples is removing the fish eggs and larvae and identifying the eggs of the target species. The second step is assigning each egg to a stage of embryonic development and counting the number of eggs of each stage for each plankton sample. Once all the samples have been staged, ages are assigned separately to each sample of the staged eggs based on a stage/temperature/age key, station temperature, and time of station occupancy. This procedure is subject to a number of sources of error that should be kept in mind and evaluated for each application.

INTRODUCTION

The daily production of eggs spawned into the sea is estimated by regressing a mortality model to density data on the number of eggs at age derived from plankton samples. To generate the egg density data, the fish eggs (and larvae) of the target species must be sorted from the plankton samples, staged on the basis of embryonic development, and aged. This process can begin as soon as the plankton samples have been transferred to the laboratory. At the Southwest Fisheries Center (SWFC), this procedure for the eggs and larvae of northern anchovies is carried out by three separate groups.

SORTING

The method of sorting eggs and larvae from the CalVET[2] samples are similar to the procedures outlined by Kramer et al. (1972) and Smith and Richardson (1977). The volume of plankton in CalVET samples was not measured. In this survey, plankton volumes were quite small because of the size of the CalVET net and the short duration of the tow and were not necessary for estimation of spawning biomass.

The plankton samples must be sorted by personnel trained in the identification of fish eggs and larvae particularly of those species from the survey area. Sorters are responsible for cross-checking the inside and outside labels of each sample jar as it is processed. It is critical that sample identification numbers written on all data forms filled out by the sorters match those on the sample jar labels. Sorters are responsible for picking out the eggs and larvae for all species and identifying the target species. At the SWFC, eggs and larvae of the target species are placed in 2-dr vials, filled with diluted Formalin, capped, and labeled with station identification numbers. Additional station data to be recorded on the staged egg data forms are water temperature and time of collection. These data are necessary for ageing the staged eggs at a later date.

Depending on the objectives of a particular survey, other species may also be sorted. Egg counts and possibly larval lengths of the target species are recorded on staged egg data forms. Sorting time per sample depends on the volume and quality of plankton and the quantity of ichthyoplankton in the samples. A sorter at the SWFC can process about eight CalVET samples per day. If processing of the plankton samples is on a strict schedule as it is at the SWFC, then it is advisable to sort first the samples collected at stations with the highest expected density of eggs.

STAGING

The second step in processing eggs of the target species is the assigning of an embryonic developmental stage to each egg. More training, experience, and time are required to accurately stage the embryonic development of fish eggs. The 2-dr vials containing sorted eggs of the target species and the respective staged egg data forms are turned over to a second group responsible for staging eggs at the SWFC.

Working with each sample separately, the technician pipettes or empties the eggs in a vial into a petri dish and sorts the eggs under a binocular dissecting scope into groups for each standardized developmental stage that spans incubation from time of fertilization to hatching, as described in Moser and Ahlstrom (1985). Eggs

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[2]For a description of the CalVET net, see Smith et al. (1985).

with disintegrated embryos are often difficult to stage and require special attention (see Moser and Ahlstrom 1985). In some samples, as much as 50% of the eggs could be disintegrated and difficult to stage. The number of eggs in each stage group are counted and recorded on the staged egg data sheet. The number of eggs for which it is impossible to assess the embryonic condition must be recorded also. The sum of the number of eggs staged should equal the earlier sorter's count. If not, then all groups should be recounted and any discrepancies accounted for and noted. When the staging of a sample is completed, all the eggs must be returned to the vial and the sample archived in a storage area.

AGEING

Once all the station samples have been staged, the completed staged data forms can be turned over to a team which assigns ages to the staged eggs. Although this group of two or three people need not be skilled in identification of eggs and larvae or egg stages, they must understand the daily spawning cycle and the effect of temperature on development rate. It is recommended that each team member age the eggs for all stations independently of the others. This serves as a check of potential ageing errors and biases that could result from subjective decisions on likely ages of eggs for which the day of spawning is not obvious.

Assigning ages to eggs based on the stage of embryonic development is a relatively straightforward procedure if spawning occurs within a brief time interval during the 24-h daily cycle and if the duration of egg stages is less than a day for the range of temperatures observed during the survey. If either one of these conditions is not the case in any particular application, then the procedure described here will have to be modified in order to estimate daily rate of egg production in the sea.

Before the ageing step can begin, the team must specify the hour of peak spawning within the daily cycle to determine time zero for egg development. This can be determined from the time of day that newly fertilized eggs appear in the egg collections and from observations on the spawning behavior or condition of the adults. A stage/temperature/age key as described in Lo (1985, fig. 2) must be available for the target species of the survey. This key represents embryonic growth curves for each developmental stage, relating time to develop with temperature. From these curves, the time or age from fertilization to time of collection can be estimated given the stage of development and station surface temperature, which is assumed to be the temperature at which the egg was incubated. The age of an egg can be calculated by estimating its age in whole days and then adding on the portion of a day that has elapsed between peak spawning hour and the time of day the sample was collected. For northern anchovies off California, the peak spawning hour is set at 2200. Thus eggs need be aged only to the nearest day and then adjusted for the portion of the day between sampling and peak spawning.

The benefit of having all spawning occur in the same short interval each day is that the ages of the eggs within each sample are separated by 24-h increments. Because the duration of any particular stage is much less than 24 h, the distribution of eggs of a single sample over the different developmental stages should form distinct groups or modes with unrepresented stages separating each group. One can then assume that each group is separated by 24 h. This pattern is extremely helpful in assigning whole-day ages to groups of eggs within a sample. However, this pattern becomes blurred for samples taken from colder temperatures where the duration of each stage is longer and the modes or groups of eggs may overlap. This pattern is also less clear for the advanced stages because the ages are assigned with less precision. The longer the incubation time, the more inflated the variance in the age key becomes as the effects of variability in growth rate and environmental factors accrue.

These two problems are partially avoided by truncating the data series and eliminating the eggs older than a specific age. The primary reason for doing this is to avoid the time period when eggs begin to hatch into larvae and are no longer available for sampling. Truncation of egg density data for eggs older than the age at which the onset of hatching is expected for the warmer temperatures encountered on the survey insures that mortality is the only process contributing to the decline in egg abundance. This also eliminates eggs for which the procedure of assigning ages has the greatest uncertainty.

For the northern anchovy surveys conducted by the SWFC, time was saved by taking temperture data at the surface only, at most stations. The recorded temperature data represent the maximum incubation temperatures that eggs would likely encounter in the upper mixed layer, where anchovy eggs are found. In a few cases, eggs could have been assigned to either one of two spawning days based on the age key. In these cases, the older age was assigned based on the assumption that the egg was probably incubated at a temperature colder than the surface waters. However, the majority of eggs closely corresponded to the predicted age suggested by the embryonic growth curves and by the separation of egg-stage modes.

Plankton samples collected during the spawning hours will undersample the new eggs until spawning is completed for the daily spawning period. To avoid underestimating daily egg production from the mortality model, egg densities of the youngest ages exclude samples collected during the spawning period. For northern anchovies this period was between 1800 and 0200. These samples do provide data on eggs older than approximately one-half day (for further discussion see Picquelle and Stauffer 1985).

After assignment of ages by all team members working independently of one another, the staged egg data forms were sorted into convenient time and temperature strata. The team as a group examined the data forms for each stratum for consistency among samples in the assigned age of each stage. At this time, discrepancies between the ages assigned by different team members and among stations with similar conditions were discussed and rectified. This final comparison is very important to minimize any differences among the team, to reduce errors, and to insure that the determinations from the age key are consistent throughout the ageing exercise.

The ageing step can be automated using a computer. Based on the stage/temperature/age key, a reference table can be constructed and included in a computer program. The table should contain the estimated ages of eggs for each of the 11 stages at temperatures ranging from 10° to 22°C, sampled at any time throughout a 24-h period. Ages are assigned to stages according to the reference table.

The advantages of the automated system are twofold. 1) It saves time: the current manual system requires about 1 wk of manpower to age the eggs, enter the data, and build the data files for the estimation of daily egg mortality and egg production; the automated system requires one-half day at most to process the staged eggs and produce a daily egg mortality curve. 2) It standardizes the method: the automated system eliminates the subjectivity of human judgment and variation resulting from operator error, thus the accuracy of the egg production and the egg mortality estimate can be improved (see Lo 1985).

ASSUMPTIONS AND SOURCES OF ERROR

1) Anchovy eggs are easily distinguished because of the oblate spheroid shape. As a result, the sorting procedure for anchovy eggs is quite accurate and relatively efficient. The sorting of spherical eggs of other species may require more skill and resorting to check for accuracy if there is a potential confusion with other species.

2) Often a high fraction of the fish eggs have disrupted or disintegrated embryos. In these cases, the developmental stage must be determined by additional criteria. For the northern anchovy example, it was assumed that this condition occurred during the plankton tow and that eggs in this category had a similar mortality history. The validity of this assumption should be examined in each application of the egg production method.

3) The assumption that 100% of the eggs are retained by the plankton net should be tested. Also, the catching process and fixation may stimulate the eggs to hatch.

4) The mortality model assumes that all egg stages including unfertilized eggs have the same rate of mortality. If unfertilized eggs make up only a small fraction of the total eggs, and if they persist in the water column for <1 d, then it is probably sufficient to assign them as 1-d-old eggs. This ignores the bias in the estimate of daily egg production created by the different mortality rates of unfertilized eggs and the inability to age unfertilized eggs. On the other hand, if unfertilized eggs are not an insignificant fraction of the total, then the egg production mortality model must be modified to account for them.

5) The ageing of staged eggs is facilitated by a short spawning interval within a daily period. Assigning ages to embryonic stages will become less reliable as the spawning interval makes up a greater fraction of the daily period. Stage durations of <12 h at the usual incubation temperature facilitates the assigning of daily ages to staged eggs. If embryonic development is slow, which is often the case at low temperatures, then there is a good possibility that eggs at a single stage could have resulted from more than a single spawning episode. If this occurs, then the arbitrary stages described here must be divided further.

6) The duration of an egg stage is a function of the incubation temperature. The measured temperature at a plankton station may not be the actual temperature at which the collected eggs were incubated within the water column. The temperature measurement must be representative of the temperature at which the majority of the eggs incubate. This requires a study of the vertical distribution of eggs compared with the temperature depth profile.

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Staging Anchovy Eggs

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ABSTRACT

The developmental period of northern anchovy eggs is divided into eleven stages. These stages are defined by structural criteria chosen from the sequence of morphological changes which occur during embryogenesis. Samples of eggs, preserved in 3% formaldehyde solution, are examined under a dissecting microscope using soft transmitted light. In Stage I eggs, cell division has not yet begun and the cytoplasm appears as a cap at one pole of the elliptical yolk mass. Cell division occurs during Stage II to produce a blastodisc. At Stage III the size of the individual blastomeres is greatly reduced and the blastodisc has the appearance of tissue. Stages IV, V, and VI are defined by the fraction of the yolk mass covered by the blastoderm. The proportion of tail length to head length defines Stages VII and VIII, and the proportion of tail length to yolk mass length defines Stages IX, X, and XI. The procedure for staging eggs with disintegrated embryos is described.

INTRODUCTION

The eleven egg stages used since 1980 in egg production biomass estimates are modified from stages established by Ahlstrom (1943) for the Pacific sardine, *Sardinops sagax*. Northern anchovy eggs differ markedly from those of the Pacific sardine in size, shape, and in the absence of an oil globule. The strongly elliptical eggs of *Engraulis mordax* measure 1.23-1.55 mm at the major axis and 0.65-0.82 mm at the minor axis (Bolin 1936), whereas developing sardine eggs range from 1.35 to 2.05 mm with a mean of 1.70 mm (Ahlstrom 1943). The sardine embryo, however, is only slightly larger than the embryo of the anchovy since the wide perivitelline space of the sardine accounts for almost half the total egg diameter. Also, the rates of development of major embryonic features and organ systems are comparable in the two species.

METHODS

Eggs are sorted from the plankton samples as described by Stauffer and Picquelle (1985) and preserved in 3% buffered Formalin in 2-dr vials. For staging, each sample is placed in a watch glass with water and examined under a dissecting microscope, using the microscope mirror to produce a soft transmitted light. A direct light source does not allow one to distinguish fine structures of the embryos.

Most samples contain several groups of eggs representing widely separated stages from several days' spawnings. An initial cursory examination allows one to sort most of the eggs into these several subgroups, which can then be assigned to stages. The stages are based on structural criteria chosen from the sequence of morphological changes which occur during embryogenesis. These are described below and illustrated in Figures 1 and 2.

EGG STAGES

Stage I

Cell division has not yet begun. In intact eggs the cytoplasm of the single cell appears as a clear hemisphere at one pole, easily differentiated from the yolk mass which is divided into granules. The cytoplasm may be displaced to other locations around the periphery of the yolk mass, but there is usually some accumulation at one pole, which allows the stage to be identified.

Stage II

This begins with the division of the single cell into two cells or blastomeres. The division is first noticeable when a furrow develops in the middle of the cytoplasmic cap. Small bubble-like structures (probably artifacts) are often visible along the furrow and help identify it. The next cleavage plane is at right angles to the first, and subsequent synchronous divisions in both meridional and latitudinal planes produce a hemispherical mound of cells, termed the blastodisc. After the 5th or 6th division, the blastodisc has a berry-like appearance, the so-called "mulberry stage," and with subsequent divisions the blastomeres become increasingly smaller and more difficult to distinguish individually. During a certain phase of the early divisions the blastomeres are about the same size as the yolk granules. If the blastodisc and yolk mass become disrupted during collection or preservation, the blastomeres may become distributed among the yolk granules. They may be distinguished from one another since they have different refractive indices and the blastomeres appear darker when viewed with transmitted light.

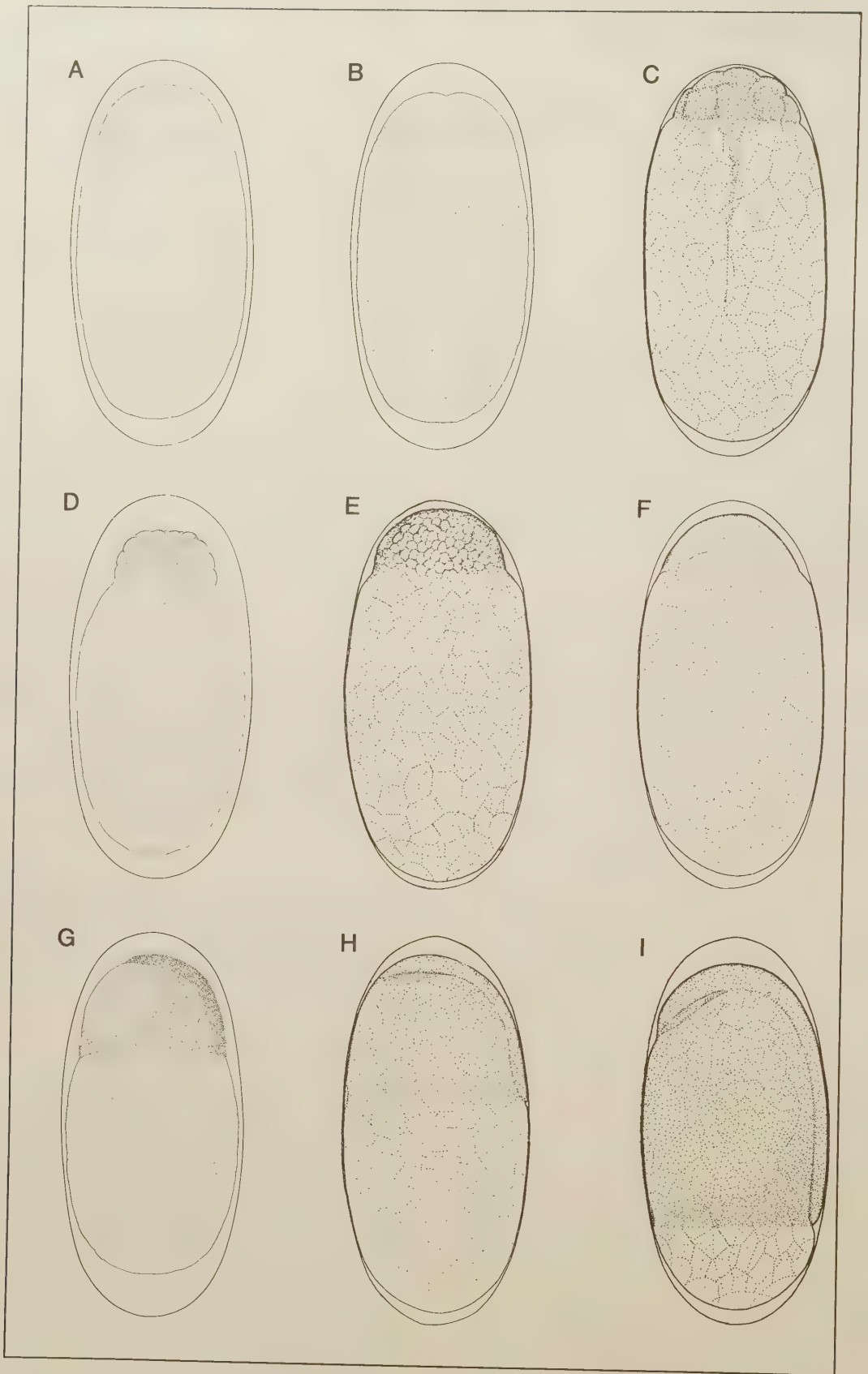

Figure 1.—Stages of northern anchovy eggs. A. Stage I; B. Stage II (2 cells); C. Stage II (16 cells); D. Stage II ("Mulberry"); E. Stage II (late); F. Stage III (mid); G. Stage III (late); H. Stage IV (mid); I. Stage V (mid). Original illustrations of A, B, D, G, by G. Mattson; C, E, F, H, I from Bolin (1936).

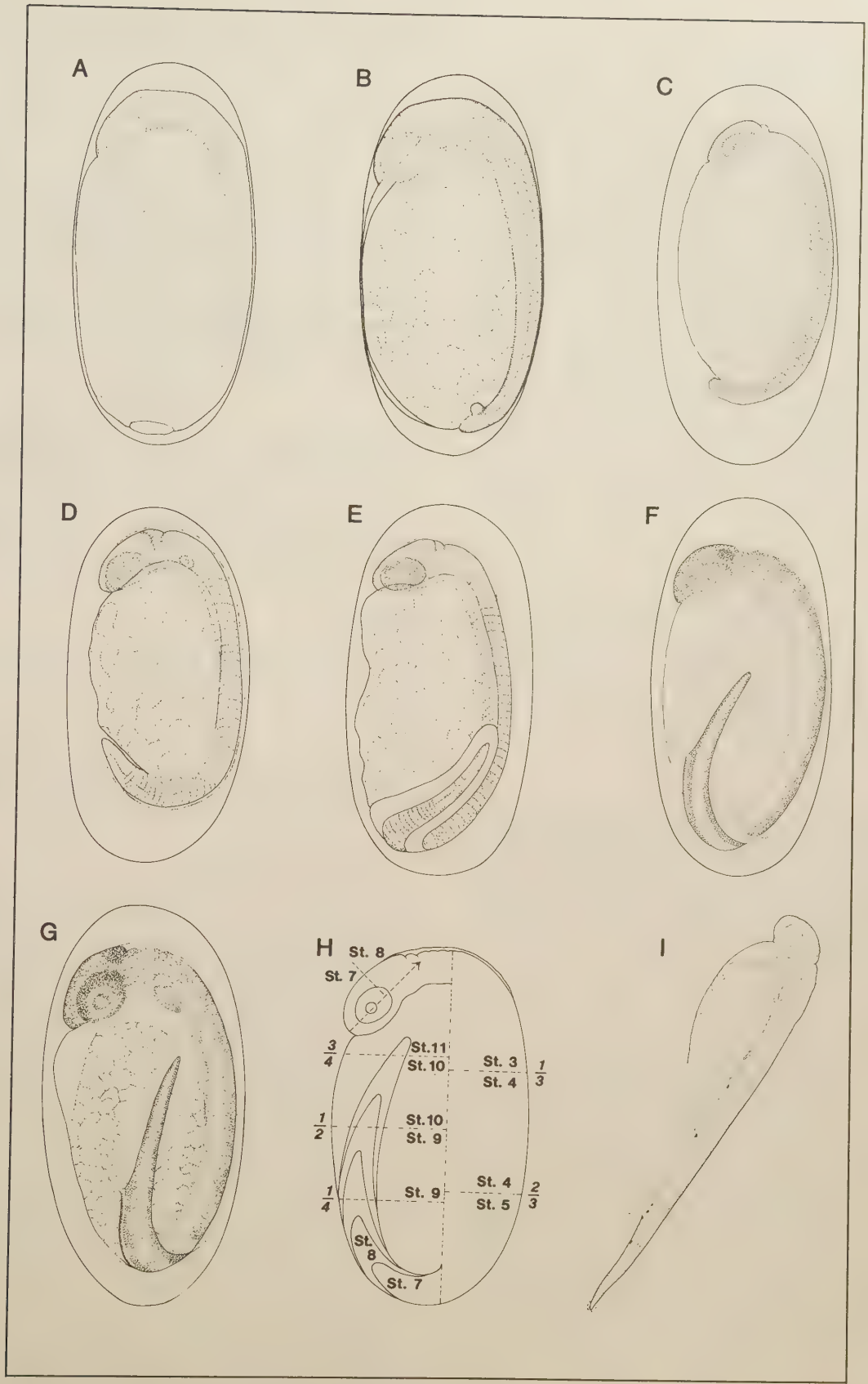

Figure 2.—Stages of northern anchovy eggs. A. Stage V (late); B. Stage VI; C. Stage VII; D. Stage VIII; E. Stage IX; F. Stage X; G. Stage XI; H. Diagrammatic egg showing relationship of epiboly and tail length to stage; right side indicates fraction of the yolk mass covered by the blastoderm in Stages III, IV, and V; left side shows tail length/head length proportions which define Stages VII and VIII and tail length/yolk mass length proportions which define Stages IX, X, and XI; I. Newly hatched anchovy. Original illustrations of A, C, and F by G. Mattson; B from Bolin (1936); original illustrations of D and E by H. Orr; original illustration of G by G. Moser; H prepared by B. Sumida; I from Kramer and Ahlstrom (1968).

Stage III

Ahlstrom (1943) defined this stage in sardine eggs as beginning with the appearance of the segmentation cavity. The segmentation cavity of teleost eggs is the space formed between the blastodisc and the yolk mass during late cleavage. In most anchovy eggs in our collections the blastodisc is shrunken and somewhat cup-shaped and consequently the segmentation cavity, which is a delicate structure, is obliterated. We have found it preferable to define the beginning of Stage III on the basis of the appearance of the blastoderm, i.e., when it has the appearance of tissue rather than of a collection of individual cells. This stage marks the beginning of gastrulation. The margin of the blastodisc becomes slightly thickened and is termed the germ ring. At one region of the germ ring the thickening extends inward to form the embryonic shield, which defines the future axis of the embryo. Gastrulation proceeds by further proliferation and downward movement of cells in the region of the germ ring by a process known as epiboly. Simultaneously, proliferation and inward migration (emboly) of cells from the margin of the embryonic shield produce the organ-forming cell layers of the primordial embryo. At the end of Stage III the germ ring is one-third down the yolk mass and the bilateral nature of the primordial embryo is apparent.

Stage IV

At the beginning of this stage the germ ring has enclosed one-third of the yolk mass and the embryo is beginning to form along the median region of the embryonic shield. At the end of this stage, defined by the germ ring enveloping two-thirds of the yolk, the head region of the embryo is becoming apparent.

Stage V

This stage begins with the germ ring two-thirds down the yolk and ends with the closure of the blastopore and the complete enclosure of the yolk by the cellular sheath of the embryo. The stage is characterized by rapid differentiation resulting in the formation of several somites in the midregion of the embryonic axis, development of the notochord which can be seen from a dorsal viewpoint, and differentiation of the optic vesicles from the brain.

Stage VI

This stage begins with the closure of the blastopore and ends when the tail starts to separate from the yolk mass. The embryonic sheath of cells is extremely thin and, in some samples, it may be difficult to determine the point of blastopore closure. In these cases the event can be estimated from the position of the caudal terminus of the embryonic axis, since it grows toward the pole with the edge of the cellular sheath. Initially the caudal region lies flat against the polar region of the yolk, then gradually thickens and becomes more rounded at the tip until it is clearly separate from the yolk. During this stage the somites are apparent along the entire body axis (except at the caudal portion), the lens primordium appears in the eye, and the regions of the brain begin to differentiate.

Stage VII

At the beginning of this stage the tip of the tail free from the yolk is broadly rounded, then begins to narrow as it elongates. The notochord extends almost to the tip and the finfold is just becoming visible. At the end of this stage the length of the free tail is one-half the length of the head. For this purpose, head length is considered the distance from the tip of the snout to the back of the cerebellum (see Fig. 2H). Relative tail length is the criterion for each remaining stage.

Stage VIII

This stage begins when the free tail length is greater than one-half the head length and ends when tail length equals head length. The tail becomes pointed during this stage and begins to bend away from the axis of the body, to the right or left side. The curvature of the tail generally increases with development, but is subject to individual variability (Fig. 2). Judgement is required in compensating for curvature in estimating relative tail length; however, accuracy and precision increase rapidly with practice.

Stage IX

This stage begins with the tail extending one-quarter the length of the yolk sac and ends when it reaches one-half the yolk sac length. The gut is now apparent along the ventral surface of the tail, and its terminal section passes through the finfold which is now considerably wider than in the previous stage. The pectoral fin buds appear as lateral thickenings as do the otic vesicles.

Stage X

This stage starts when the tail is one-half the length of the yolk sac and ends when it reaches three-quarters of the yolk sac length.

Stage XI

This is the final stage before hatching and is defined by a tail length greater than three-quarters of the length of the yolk sac.

Disintegrated (Dis) Eggs

Eggs with embryos in various states of disintegration are found in many samples, and some samples contain a large proportion of disintegrated eggs. Despite some preliminary field and laboratory experiments, we are not able to determine whether disintegration is caused by net damage, fixation, mortality prior to capture, or some combination of these. The sampling design requires that all eggs be assigned a stage regardless of condition. Empty egg shells and eggs which have no identifiable morphological features are assigned the "Dis" (disintegrated) category. These are later assigned a stage, by pro rating, during the aging procedure (see Lo 1985). With detailed examination, eggs containing damaged or shrunken embryos can be staged. Usually they are part of a mode which is present in the sample and thus can be staged by comparison with the groups of intact eggs. In the early-stage disintegrated eggs, the blastomeres and yolk granules are intermingled but can be distinguished since the former appear darker in transmitted light. In eggs beyond Stage III, the embryos are rarely disassociated but may be distorted or shrunken and oriented abnormally because of the disintegration of the yolk mass. Despite their condition, these embryos have the morphological features described for intact embryos and can be staged, although this requires more attention to morphological details; particularly useful are careful observations on the degree of differentiation of myomores, median finfold, and head structures such as eyes, brain lobes, and otic vesicles.

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A Model for Temperature-Dependent Northern Anchovy Egg Development and an Automated Procedure for the Assignment of Age to Staged Eggs

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ABSTRACT

A functional relationship between age and developmental stage of northern anchovy, *Engraulis mordax*, eggs for various temperatures was determined based upon data collected in two laboratory experiments. The age of an egg was found to be a mixed exponential and power function of temperature and stage. An automated procedure was also developed using a reference table to assign age to staged eggs for any given time of tow and sea temperature ranging from 10° to 22°C. A comparison between the manual system and the automated procedure was made based on 1980-83 data, and the difference was found to be minimal.

INTRODUCTION

In the first section of this paper, I discuss the modelling of temperature-dependent anchovy egg development based on data from two laboratory experiments conducted at the Southwest Fisheries Center (SWFC). This temperature-dependent egg development curve is used to assign ages to the field-collected eggs after they are staged according to their anatomical development. In the second section, I discuss an automated procedure for accomplishing the assignment of ages.

TEMPERATURE AND DEVELOPMENT

Both laboratory and field-collected anchovy egg data indicate that the developmental rate of eggs is temperature-dependent. The higher the temperature, the shorter the time required to reach a given stage. The relationship between the age of eggs and temperature for a given stage has been studied in the past (Zweifel and Lasker 1976; Zweifel pers. comm.[1]) and a Gompertz growth curve fitted to the laboratory egg development data of both anchovy and sardine.

To improve our knowledge of the relationship between temperature and development of anchovy eggs and yolksac larvae, in 1981 a laboratory experiment was conducted at three temperatures (13.5°, 15.0°, and 16.5°C) using eggs from a captive brood stock (Lo 1983). These egg data were used along with those collected at temperatures ranging from 13.8° to 20.8°C for stages III, VI, VIII, IX, and XI in an earlier experiment to develop a revised model for expressing the development of anchovy eggs at a given temperature (Table 1). Stages are described by Moser and Ahlstrom (1985), and procedures for sorting and staging eggs by Stauffer and Picquelle (1985).

Table 1.—Average age (in hours.minutes) of northern anchovy eggs for each of 10 developmental stages (Stage I not observed) at various temperatures (°C) from laboratory experiments conducted at the SWFC.

	Temperature (°C)								
	(Lo 1983)			Zweifel and Lasker (1976)					
Stage	13.9	15.2	16.2	13.8	15.2	16.6	18.0	19.4	20.8
II	6.82	6.42	6.40						
III	16.36	13.55	13.14	20	15	10	9	8	6
IV	24.91	21.12	20.18						
V	35.88	30.7	25.56						
VI	44.63	39.24	33.20	42	35	26	24	21	9
VII	49.64	47.13	38.56						
VIII	52.5	52.07	43.64						
IX	61.1	56.28	50.48	58	50	39	35	33	28
X	66.09	62.63	54.78						
XI	69.79	65.65	56.10	78	65	55	44	39	35

MODEL DEVELOPMENT AND RESULTS

To choose an appropriate model for the relationship between age and temperature for a particular stage, I examined the age-temperature relationship empirically for stages III, VI, VIII, IX, and XI, because data on these stages were available for a wide temperature range (data for stages VIII and IX were combined to increase the sample size).

[1] J. R. Zweifel, Southeast Fisheries Center, National Marine Fisheries Service, NOAA, 75 Virginia Beach Drive, Miami, FL 33149, pers. commun. July 1983.

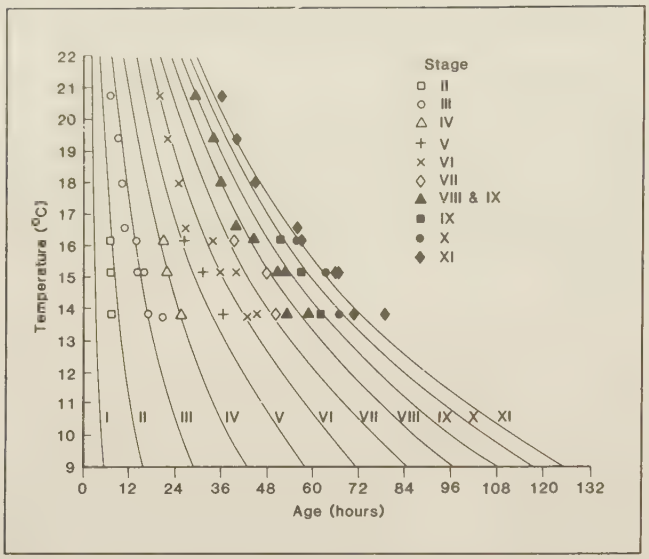

Figure 1.—Observed (denoted by various symbols) and predicted ages (smooth lines) of time to the *i*th stage as a function of temperature (°C) based on egg development data collected from two laboratory experiments conducted at SWFC. The predicted values are computed from Equation (4).

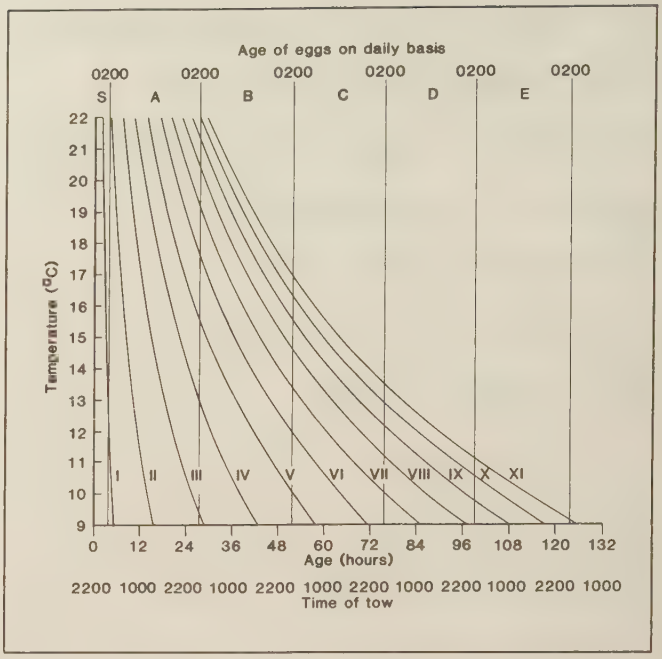

Figure 2.—Function relationship of average age of eggs for the *i*th stage based upon time of tow and sea surface temperature (°C).

For all four stages, the exponential function fitted the data well, i.e.,

$$y_{i,t} = a_i e^{b_i t} \quad (1)$$

where $y_{i,t}$ is the average age of the *i*th-stage anchovy eggs at temperature t°C. The coefficients (a_i,b_i) are for the *i*th stage only. The initial fitting of the model was accomplished by linear regression of the natural logarithm of the ages on the temperatures ($\ln y_{i,t} = \ln a_i + b_i t$). The coefficient of determination (R^2) ranges from 0.82 to 0.98:

Stage	$\ln a_i$	b_i	R^2
III	5.02	−0.155	0.95
VI	5.50	−0.126	0.82
VIII, IX	5.54	−0.107	0.95
XI	5.88	−0.113	0.98

Next, the development data were examined for a given temperature where ages were available for most of the stages. In this analysis, only the latest laboratory data were included because the other data set had only four or five egg stages. The temperatures used were 13.9°, 15.2°, and 16.2°C (mean temperature experienced by all egg stages). For each of the three temperatures, the development curves were more complicated than time-to-stage curves described above. A simple exponential function was not sufficient. Instead, the function was a combination of an exponential and a power function:

$$y_{i,t} = a_t e^{b_t i} (i)^{c_t} \quad (2)$$

All the coefficients are temperature specific; a_t, b_t, and c_t are for temperature t°C. Linear regressions of the natural logarithms of average age on stage ($\ln y_{i,t} = \ln a_t + b_t \cdot i + c_t \ln(i)$) show an excellent fit with R^2 ranging from 0.96 to 0.99 for each of the three temperatures:

Temperature (°C)	$\ln a_t$	b_t	c_t	R^2
13.9	0.717	−0.206	2.4	0.96
13.5	0.731	−0.153	2.13	0.96
16.2	0.793	−0.206	1.94	0.99

In order to include all the existing data on anchovy egg development in a single model, Equations (1) and (2) were combined into one equation:

$$y_{i,t} = a\, e^{(bt+ci)} i^d \quad (3)$$

where the coefficients a, b,c, and d were common for all stages and all temperatures. Equation (3) was then fitted to all temperature-specific egg development data (Table 1). The resulting model is:

$$y_{i,t} = 16.07\, e^{-(0.1145t+0.0098i)} i^{1.74}. \quad (4)$$

The observed and predicted ages of anchovy eggs were plotted in Figure 1.

For each of the 11 stages, the average age of anchovy eggs can be estimated from Equation (4) for a given temperature. A family of 11 temperature-age curves was used as the key to determine the ages of anchovy eggs collected in the field in conjunction with the time of tow and sea surface temperature, assuming 1800-0200 was the spawning time with 2200 as the peak (Fig. 2).

To use Figure 2 to assign age to anchovy eggs after they are staged, the following is done. First, time zero was assumed to be 2200, the midpoint of the daily spawning period. All the eggs observed between 1800 and 0200 were classified as newly spawned eggs (S). Eggs that fall in the consecutive 24-h intervals from 0200 to 0200 are classified as day 0, day 1, day 2, and day 3 eggs (or A-day, B-day, C-day, D-day eggs), depending on the time of tow and the sea surface temperature. For example, the eggs collected from a tow taken at 1800 h at 16°C should have the age assignment as below (theoretically no stage II should be observed):

Stage	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Age	S	—	A	A	A	B	B	B	B	B	B,C

The above assignment of ages to staged eggs is on a daily basis. With the information of time of tow, the age of eggs can be expressed in hours, e.g., A-day eggs are 20 h old (20 h from 2200) and B-day eggs are 44 h old in this example.

AN AUTOMATED PROCEDURE

The automated procedure for assigning an age to a staged anchovy egg is a FORTRAN computer program (STAGEAGE) coded for VAX[2] computer. It takes input data of staged eggs along with sea temperature and time of tow and assigns ages to eggs according to a reference table (see Table 3). This reference table contains the estimated ages of eggs for each of the 11 egg stages (see Moser and Ahlstrom 1985) at temperatures ranging from 10° to 22°C for any time throughout a day. In the following paragraphs, I describe the construction of the reference table.

The estimated ages ($y_{i,t,k}$) in the reference table are the average ages ($y_{i,t}$) computed from Equation (4) adjusted for the time of tow (k) assuming that the peak spawning time is 2200. If the eggs are observed before their expected time, an age younger than $y_{i,t}$ will be assigned. Otherwise, an older age will be assigned. The basic formula of determining the age of eggs ($y_{i,t,k}$) is

$$y_{i,t,k} = y_{i,t} + k - \hat{T} \qquad (5)$$

[2]VAX is a trademark of Digital Equipment Corporation.

where $y_{i,t}$ is from Equation (4), k is time of tow, and $\hat{T}$ is the expected time of observing stage i eggs. $\hat{T}$ is obtained from the peak spawning time (st) (=2200 for northern anchovy) and average age ($y_{i,t}$):

$$\hat{T} = \text{remainder of } (y_{i,t} + st)/24.$$

The basic formula is used for samples taken between $\hat{T}$-G and $\hat{T}$+G h. The quantity G is chosen to be 2× the standard deviation of ages within stage (sd). If $y_{i,t,k}<2\times sd$, G is set to be equal to $y_{i,t,k}$, or

$$G = \begin{cases} 2\times sd & \text{if } y_{i,t,k}>2\times sd \\ y_{i,t,k} & \text{otherwise.} \end{cases}$$

The average standard deviation over three temperatures (13.9°, 15.2°, 16.2°C) is between 2 and 5 h.

Stage	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
sd (h)	—	2	4	4	4	4	3	3	5	3	3

The average age ($y_{i,t}$) and the expected time of tow ($\hat{T}$) for each stage and temperature combination are given in Table 2. The assignment of ages of eggs as a function of the time of tow (k) and sea surface temperature (t) is computed as below:

$$y_{i,t,k} = \begin{cases} y_{i,t}-G & \text{if } k<\hat{T}-G \\ y_{i,t}+k-\hat{T} & \hat{T}-G<k<\hat{T}+G \\ y_{i,t}+G & \hat{T}+G<k. \end{cases} \qquad (7)$$

Notice Equation (7) is a modified version of Equation (5).

Table 2.—Average age and expected time of tow for each combination of stages (i) and temperatures (t) ranging from 10° to 22°C.

Temp. (°C)	Stage I	II	III	IV	V	VI	VII	VIII	IX	X	XI
	Average ($y_{i,t}$) (in hours.minutes)										
10	4.23	13.16	24.21	36.25	48.40	60.36	71.51	82.11	91.27	99.36	106.36
11	3.54	11.50	21.43	32.28	43.24	54.03	64.05	73.17	81.34	88.50	95.04
12	3.29	10.33	19.22	28.57	38.43	48.12	57.09	65.22	72.44	79.13	84.47
13	3.06	9.24	17.16	25.50	34.31	42.59	50.58	58.17	64.52	70.39	75.36
14	2.46	8.23	15.24	23.02	30.47	38.20	45.27	51.59	57.51	63.00	67.26
15	2.28	7.29	13.44	20.32	27.27	34.11	40.32	46.22	51.36	56.11	60.08
16	2.12	6.40	12.15	18.19	24.29	30.29	36.09	41.21	46.01	50.07	53.38
17	1.58	5.57	10.55	16.20	21.50	27.11	32.14	36.52	41.02	44.41	47.49
18	1.45	5.18	9.44	14.34	19.28	24.15	28.45	32.53	36.36	39.51	42.39
19	1.34	4.44	8.41	13.00	17.22	21.38	25.38	29.19	32.38	35.32	38.02
20	1.24	4.13	7.45	11.35	15.29	19.17	22.52	26.09	29.06	31.42	33.55
21	1.15	3.46	6.55	10.20	13.49	17.12	20.23	23.19	25.57	28.16	30.15
22	1.06	3.21	6.10	9.13	12.19	15.20	18.11	20.48	23.09	25.13	26.59
	Expected time of tow ($\hat{T}$) (in hours.minutes)										
10	2.23	11.16	22.21	10.25	22.40	10.36	21.51	8.11	17.27	1.36	8.36
11	1.54	9.50	19.43	6.28	17.24	4.03	14.05	23.17	7.34	14.50	21.04
12	1.29	8.33	17.22	2.57	12.43	22.12	7.09	15.22	22.44	5.13	10.47
13	1.06	7.24	15.16	23.50	8.31	16.59	0.58	8.17	14.52	20.39	1.36
14	0.46	6.23	13.24	21.02	4.47	12.20	19.27	1.59	7.51	13.00	17.26
15	0.28	5.29	11.44	18.32	1.27	8.11	14.32	20.22	1.36	6.11	10.08
16	0.12	4.40	10.15	16.19	22.29	4.29	10.09	15.21	20.01	0.07	3.38
17	23.58	3.57	8.55	14.20	19.50	1.11	6.14	10.52	15.02	18.41	21.49
18	23.45	3.18	7.44	12.34	17.28	22.15	2.45	6.53	10.36	13.51	16.39
19	23.34	2.44	6.41	11.00	15.22	19.38	23.38	3.19	6.38	9.32	12.02
20	23.24	2.13	5.45	9.35	13.29	17.17	20.52	0.09	3.06	5.42	7.55
21	23.15	1.46	4.55	8.20	11.49	15.12	18.23	21.19	23.57	2.16	4.15
22	23.06	1.21	4.10	7.13	10.19	13.20	16.11	18.48	21.09	23.13	0.59

Thus, the age for eggs of stage i observed at temperature t is always within $y_{i,t} \pm G$ (i.e., between two standard deviations of age within a stage). Equation (7) eliminates the possibility of assigning either too young or too old an age to eggs that were sampled at the time which is more than G hours before or after the expected time of tow. Occasionally, the eggs are taken 12 h away from the expected time of spawning. These eggs can be either the youngest or the oldest eggs for that stage. In this case, the maximum and minimum ages are then randomly assigned to those eggs because there is no way of distinguishing eggs between the young and old ages within stage.

The reference table for stage IV anchovy eggs is given (Table 3). In the table, the first column is the time of tow (01-24 h). The rows corresponding to 25 h are the maximum ages used for random selection of ages for eggs observed at 12 h away from the expected time. Row 1 contains temperatures ranging from 10° to 22°C. Each entry from row 2 and on is the estimated age for a combination of time of tow and sea temperature. For a given temperature, and time of tow, ages are assigned to each stage according to the reference table. The reference table for all stages is included in FORTRAN program STAGEAGE (Hewitt et al. 1984).

Use of Computer Program STAGEAGE

Input Data File—The input data file of eggs should include tow identification (ID) information, e.g., cruise number, location (line and station for CalCOFI survey), stratum, weighting factor (Hewitt et al. 1984), tow number, tow time, sea surface temperature, number of eggs for each stage, and total number of eggs. Variables in tow ID and total eggs are useful for data checking. The other variables are essential for estimating the ages of eggs (Table 4).

Output Data Files—Several output data files are available from STAGEAGE. STAGEAGE can be used not only to assign ages to staged eggs, but also to stratify the survey area into regions as desired. Regions are defined as area between line numbers. The user can choose output data file(s) and the number of regions in the execution instructions (see next section). The major output data file names in logic unit numbers are FOR026.DAT, FOR029.DAT, FOR036.DAT, FOR037.DAT, FOR038.DAT (Tables 5a-e).

Table 3.—Partial listing of the reference table. Estimated age (in hours.minutes) for stage IV for each combination of time of tow (1-24 h) and temperature (10°-22°C). Asterisks (*) indicate the estimated age corresponding to expected time of tow ($\hat{T}$) (see Tables 2a, b). Row 25 is for random assignment of ages for eggs observed at 12 h before or after expected time of tow.

Time of tow (k)	Temperature (°C)												
	10	11	12	13	14	15	16	17	18	19	20	21	22
1	28.25	27.28	27.57	27.50	27.02	27.32	26.19	24.20	6.34	4.60	3.35	3.20	3.13
2	28.25	28.28	28.57*	28.50	28.02	28.32	26.19	8.20	6.34	4.60	4.35	4.20	4.13
3	29.25	29.28	29.57	29.50	29.02	28.32	26.19	8.20	6.34	5.60	5.35	5.20	5.13
4	30.25	30.28	30.57	30.50	30.02	28.32	10.19	8.20	6.34	6.60	6.35	6.20	6.13
5	31.25	31.28	31.57	31.50	31.02	28.32	10.19	8.20	7.34	7.60	7.35	7.20	7.13
6	32.25	32.28*	32.57	32.50	31.02	12.32	10.19	8.20	8.34	8.60	8.35	8.20	8.13
7	33.25	33.28	33.57	33.50	31.02	12.32	10.19	9.20	9.34	9.60	9.35	9.20	9.13*
8	34.25	34.28	34.57	33.50	31.02	12.32	10.19	10.20	10.34	10.60	10.35	10.20*	10.13
9	35.25	35.28	35.57	33.50	15.02	12.32	11.19	11.20	11.34	11.60	11.35*	11.20	11.13
10	36.25*	36.28	36.57	33.50	15.02	12.32	12.19	12.20	12.34	12.60*	12.35	12.20	12.13
11	37.25	37.28	36.57	17.50	15.02	13.32	13.19	13.20	13.34	13.60	13.35	13.20	13.13
12	38.25	38.28	36.57	17.50	15.02	14.32	14.19	14.20	14.34*	14.60	14.35	14.20	14.13
13	39.25	39.28	36.57	17.50	15.02	15.32	15.19	15.20	15.34	15.60	15.35	15.20	15.13
14	40.25	40.28	20.57	17.50	16.02	16.32	16.19	16.20*	16.34	16.60	16.35	16.20	16.13
15	41.25	40.28	20.57	17.50	17.02	17.32	17.19	17.20	17.34	17.60	17.35	17.20	17.13
16	42.25	40.28	20.57	18.50	18.02	18.32	18.19*	18.20	18.34	18.60	18.35	18.20	17.13
17	43.25	40.28	20.57	19.50	19.02	19.32	19.19	19.20	19.34	19.60	19.35	18.20	17.13
18	44.25	24.28	20.57	20.50	20.02	20.32*	20.19	20.20	20.34	20.60	19.35	18.20	17.13
19	44.25	24.28	21.57	21.50	21.02	21.32	21.19	21.20	21.34	20.60	19.35	18.20	1.13
20	44.25	24.28	22.57	22.50	22.02	22.32	22.19	22.20	22.34	20.60	19.35	2.20	1.13
21	44.25	24.28	23.57	23.50	23.02*	23.32	23.19	23.20	22.34	20.60	3.35	2.20	1.13
22	28.25	24.28	24.57	24.50	24.02	24.32	24.19	24.20	22.34	4.60	3.35	2.20	1.13
23	28.25	25.28	25.57	25.50*	25.02	25.32	25.19	24.20	22.34	4.60	3.35	2.20	1.13
24	28.25	26.28	26.57	26.50	26.02	26.32	26.19	24.20	6.34	4.60	3.35	2.20	2.13
25	44.25	40.28	36.57	33.50	31.02	28.32	26.19	24.20	22.34	20.60	19.35	18.20	17.13

FOR026.DAT contains tow ID, region, tow time, temperature, and number of eggs and age grouped by 24-h increments: 0-3, 4-27, 28-51, 52-75, and 76-99 as S, A, B, C, and D-day categories for each tow. The program assigns number 0 to an age if it is impossible to observe eggs that belong to such a day category for a given time of tow. For example, S-day eggs are never observed at 1200 (Table 5a).

FOR029.DAT contains the same data as FOR026.DAT except the age is not included and this file was formatted for the CalCOFI data base for the SWFC (Table 5b).

FOR036.DAT contains tow ID, region, tow time, temperature, and number of eggs and age for each stage by tow (Table 5c).

FOR037.DAT contains records in which at least one stage was observed outside the normal range of expected time, i.e., $\hat{T} \pm G$. This file is useful for double-checking the accuracy of data on eggs observed at unusual times (Table 5d).

FOR038.DAT contains tow ID and two variables: number of eggs and age for A, B, and C-day eggs. The day categories with age >2.5 d are excluded. Therefore, the total number of records is <3× the number of tows. This file can be used directly for regression estimates of egg production and egg mortality rates (Table 5e).

Execution Instructions—The program may be executed interactively or as a batch job. An example of a set of commands for VAX is given below with input data file FILENAME:

```
$ASSIGN FILENAME FOR025
$RUN STAGEAGE
(. . .enter 1 for each of the output files needed:FOR026,028,029,
036,037, and 038. Enter 2, otherwise.)
1 2 1 2 1 1
(enter number of regions)
3
(enter max (CalCOFI) line number for each region, separated by
space.)
826 950 1500
```

This set of commands takes FILENAME as the input file and asks for output files FOR026, 029, 037, and 038. Commands in parentheses are prompted by the machine. Other lines are inputs by the user. The input and output data files for 1983 anchovy eggs are listed in Tables 4 and 5 for illustration.

Table 4.—Partial listing of input data file for 1983 northern anchovy eggs (N8302.DAT) for the computer program STAGEAGE. (Cruise no. is 8302; 8 was deleted from printout.)

Cruise no.	Tow no.	CalCOFI line	CalCOFI stn.	Stratum	Weighting factor	Date	Tow time (h)	Temp. (°C × 10)	Egg stages											Disintegrated eggs	Total no eggs/tow
									I	II	III	IV	V	VI	VII	VIII	IX	X	XI		
302	1	733	500	0	1.000	830205	408	150	0	0	0	0	0	0	0	0	0	0	0	0	0
302	2	733	510	0	1.000	830205	545	149	0	0	0	0	0	0	0	0	0	0	0	0	0
302	3	733	520	0	1.000	830205	631	149	0	0	0	0	0	0	0	0	0	0	0	0	0
302	4	733	530	0	1.000	830205	837	148	0	0	0	0	0	0	0	0	0	0	0	0	0
302	5	733	540	0	1.000	830205	1028	146	0	0	0	0	0	0	0	0	0	0	0	0	0
302	6	733	550	1	1.000	830205	1113	148	0	0	0	0	0	0	0	0	0	1	0	0	1
302	7	733	560	0	1.000	830205	1159	148	0	0	0	0	0	0	0	0	0	0	0	0	0
302	8	733	570	0	1.000	830205	1245	150	0	0	0	0	0	0	0	0	0	0	0	0	0
302	9	733	580	0	1.000	830205	1328	148	0	0	0	0	0	0	0	0	0	0	0	0	0
302	10	733	590	0	1.000	830205	1416	147	0	0	0	0	0	0	0	0	0	0	0	0	0
302	11	733	600	0	1.000	830205	1633	147	0	0	0	0	0	0	0	0	0	0	0	0	0
302	12	741	600	0	1.000	830205	1831	146	0	0	0	0	0	0	0	0	0	0	0	0	0
302	13	741	590	0	1.000	830205	2035	147	0	0	0	0	0	0	0	0	0	0	0	0	0
302	14	741	580	0	1.000	830205	2109	147	0	0	0	0	0	0	0	0	0	0	0	0	0
302	15	741	570	0	1.000	830205	2144	147	0	0	0	0	0	0	0	0	0	0	0	0	0
302	16	741	560	0	1.000	830205	2231	147	0	0	0	0	0	0	0	0	0	0	0	0	0
302	17	741	550	1	1.000	830205	2328	146	0	0	0	0	1	0	0	0	0	0	0	0	1
302	18	741	540	1	1.000	830206	20	146	0	0	0	0	0	0	0	1	0	0	0	0	1
302	19	741	530	1	1.000	830206	113	146	0	0	0	0	0	0	0	3	0	0	0	0	3
302	20	741	520	0	1.000	830206	151	148	0	0	0	0	0	0	0	0	0	0	0	0	0
302	21	741	510	0	1.000	830206	228	149	0	0	0	0	0	0	0	0	0	0	0	0	0
302	22	741	500	0	1.000	830206	312	149	0	0	0	0	0	0	0	0	0	0	0	0	0
302	23	741	492	0	1.000	830206	417	148	0	0	0	0	0	0	0	0	0	0	0	0	0
302	24	750	485	1	1.000	830206	619	146	0	0	0	0	0	0	0	0	0	0	0	0	0
302	26	750	500	1	1.000	830206	1539	151	0	0	0	0	0	0	0	0	0	2	0	0	2
302	27	750	510	1	1.000	830206	1717	151	0	0	0	0	0	0	0	0	0	0	0	0	0
302	29	750	520	1	1.000	830206	2232	151	0	0	0	0	0	0	0	0	0	0	0	0	0
302	30	750	530	1	1.000	830206	2311	148	0	0	0	0	0	0	0	0	0	0	0	0	0
302	31	750	540	1	1.000	830207	8	143	0	0	0	0	0	0	0	0	2	0	0	1	3
302	32	750	550	1	1.000	830207	49	143	0	0	0	0	0	0	0	0	0	2	0	0	2
302	33	750	560	1	1.000	830207	147	144	0	0	0	0	0	0	0	0	0	2	0	0	2
302	34	750	570	1	1.000	830207	231	145	0	0	0	0	0	0	0	0	0	1	0	0	1
302	35	750	580	0	1.000	830207	309	144	0	0	0	0	0	0	0	0	0	0	0	0	0
302	36	750	590	0	1.000	830207	350	146	0	0	0	0	0	0	0	0	0	0	0	0	0
302	37	750	600	0	1.000	830207	441	146	0	0	0	0	0	0	0	0	0	0	0	0	0
302	38	758	600	1	1.000	830207	616	146	0	0	0	0	0	0	0	0	2	0	0	0	2
302	39	758	590	1	1.000	830207	720	146	0	0	0	0	0	0	0	0	2	0	0	0	2
302	40	758	580	1	1.000	830207	807	146	0	0	0	0	0	0	0	0	2	0	0	0	2
302	41	758	570	1	1.000	830207	852	144	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 5a.—Partial listing of output data file (FORO26.DAT) for 1983: Number of northern anchovy eggs and estimated ages (in hours) grouped by S(0-3 h), A(4-27 h), B(28-51), C(52-75 h), D(76-99 h). Disintegrated eggs are obtained by subtraction. E-day eggs (100-123 h) are not observed for northern anchovy and thus are not included in the output. (Cruise no. is 8302.)

Cruise no.	Tow no.	CalCOFI line	CalCOFI stn.	Stratum	Weighting factor	Date	Region	Tow time (h)	Temp. (°C)	No. eggs and ages (h)/day catagories										Total
										S		A		B		C		D		
										N	h	N	h	N	h	N	h	N	h	
302	17	741	550	1	1.000	830205	1	23 28	14	0.	1.	1	25.	0.	48.	0.	71.	0.	0.	1.
302	18	741	540	1	1.000	830206	1	24 20	14	0.	2	0	20.	1.	50.	0	71.	0.	0.	1.
302	19	741	530	1	1.000	830206	1	1 13	14	0	3	0.	20	3	51	0	65.	0.	0.	3
302	24	750	485	1	1.000	830206	1	6 19	14	0.	0.	0.	7	0.	37.	0.	58.	0.	0.	0.
302	26	750	500	1	1.000	830206	1	15 39	15	0.	0	0.	16	0.	41.	2	62.	0.	0.	2
302	27	750	510	1	1.000	830206	1	17 17	15	0.	2	0.	19	0.	43.	0	64.	0.	0.	0
302	29	750	520	1	1.000	830206	1	22 32	15	0.	2	0	24.	0.	48	0	66.	0.	0.	0.
302	30	750	530	1	1.000	830206	1	23 11	14	0.	1.	0.	19.	0.	48.	0.	71.	0.	0.	0
302	31	750	540	1	1.000	830207	1	24 8	14	0.	2.	0.	20	2.	50.	0	71.	0.	0.	3.
302	32	750	550	1	1.000	830207	1	24 49	14	0.	2.	0	20.	0.	49.	2	69.	0.	0.	2.
302	33	750	560	1	1.000	830207	1	1 47	14	0.	3	0	20	0.	46	2	57.	0.	0.	2.
302	34	750	570	1	1.000	830207	1	2 31	14	0.	0	0	5.	0.	34	1.	57.	0.	0.	1.
302	38	758	600	1	1.000	830207	1	6 16	14	0.	0	0	7	0.	37	2	56	0.	0.	2
302	39	758	590	1	1.000	830207	1	7 20	14	0.	0	0	7	0.	34.	2	57.	0.	0.	2
302	40	758	580	1	1.000	830207	1	8 7	14	0.	0.	0	8	0.	35	2.	58.	0.	0.	2
302	41	758	570	1	1.000	830207	1	8 52	14	0.	0	0	8.	0.	35.	0.	59.	0.	0.	0.
302	42	758	560	1	1.000	830207	1	9 34	14	0.	0	0.	10.	0.	36	0.	59.	0.	0.	0.
302	43	758	550	1	1.000	830207	1	10 39	14	0.	0	0	11.	0.	37.	2.	60.	0.	0.	2.
302	44	758	540	1	1.000	830207	1	11 22	14	0.	0	0	11	0.	38	2.	61.	0.	0.	2.
302	45	758	530	1	1.000	830207	1	12 0	15	0.	0	0	13	1.	38	1	62.	0.	0.	2.
302	46	758	520	1	1.000	830207	1	12 35	15	0.	0	0	13	0.	38	3	61.	0.	0.	3
302	47	758	510	1	1.000	830207	1	13 16	15	0.	0	0	15	0.	40	0	63	0.	0.	0
302	49	758	495	1	1.000	830207	1	14 30	15	0.	0	0.	16	0.	40	0	63.	0.	0.	0
302	50	767	480	1	1.000	830207	1	17 9	15	0.	0	0	17.	0.	43.	0.	64.	0.	0.	0
302	51	767	490	1	1.000	830207	1	18 28	15	0.	2	0	20.	1.	44	0.	64.	0.	0.	1.
302	52	767	500	1	1.000	830207	1	20 46	15	0.	2	0	23	0.	47	0.	66.	0.	0.	0
302	53	767	510	1	1.000	830207	1	22 25	15	0.	2	3	24	1.	48.	0	66	0.	0.	4.
302	54	767	520	1	1.000	830208	1	24 43	15	0.	3	0	21	0.	49	0	54	0.	0.	0.
302	55	767	530	1	1.000	830208	1	1 31	15	0	3	0	22	0.	50.	0	54	0.	0.	0.
302	56	767	540	1	1.000	830208	1	2 17	15	0.	0	0.	4	1.	28	0.	53	0.	0.	1.
302	57	767	550	1	1.000	830208	1	3 6	15	0.	0	1	5.	1.	29.	0.	53.	0.	0.	2.
302	58	767	560	1	1.000	830208	1	5 28	15	0.	0	0.	6.	1.	31	0.	54.	0.	0.	1.
302	59	767	570	1	1.000	830208	1	6 22	14	0.	0	0	7.	1.	32.	0	58.	0.	0.	1.
302	64	775	590	1	1.000	830208	1	14 14	14	0.	0	0	15	1.	38.	0.	64.	0.	0.	1.
302	65	775	580	1	1.000	830208	1	14 53	14	0.	0.	0.	15.	0.	41.	0.	64.	0.	0.	0.
302	66	775	570	1	1.000	830208	1	15 29	15	0.	0	0	16	0.	41	1.	62	0.	0.	1.
302	67	775	560	1	1.000	830208	1	16 15	15	0.	0	0.	17.	1.	42.	1	66.	0.	0.	2
302	68	775	550	1	1.000	830208	1	17 3	14	0.	0	0	18.	0.	44	0.	67.	0.	0.	0.

Table 5b.—Partial listing of output data file (FORO29.DAT). Number of northern anchovy eggs grouped by S(0-3 h), A(4-27 h), B(28-51 h), C(52-75 h), D(76-99 h), and E(100-123 h).

Cruise no.	Tow no.	CalCOFI line	CalCOFI stn.	Stratum	Weighting factor	Date	Tow time (h)	Temp. (°C x 10)	No. eggs/ day categories						Disintegrated eggs	Total no. eggs in tow
									S	A	B	C	D	E		
8302	19	741	530	1	1.00	830206	113	146	0	0	3	0	0	0	0	3
8302	24	750	485	1	1.00	830206	619	146	0	0	0	0	0	0	0	0
8302	26	750	500	1	1.00	830206	1539	151	0	0	0	2	0	0	0	2
8302	27	750	510	1	1.00	830206	1717	151	0	0	0	0	0	0	0	0
8302	29	750	520	1	1.00	830206	2232	151	0	0	0	0	0	0	0	0
8302	30	750	530	1	1.00	830206	2311	148	0	0	0	0	0	0	0	0
8302	31	750	540	1	1.00	830207	24 8	143	0	0	2	0	0	0	1	3
8302	32	750	550	1	1.00	830207	2449	143	0	0	0	2	0	0	0	2
8302	33	750	560	1	1.00	830207	147	144	0	0	0	2	0	0	0	2
8302	34	750	570	1	1.00	830207	231	145	0	0	0	1	0	0	0	1
8302	38	758	600	1	1.00	830207	616	146	0	0	0	2	0	0	0	2
8302	39	758	590	1	1.00	830207	720	146	0	0	0	2	0	0	0	2
8302	40	758	580	1	1.00	830207	8 7	146	0	0	0	2	0	0	0	2
8302	41	758	570	1	1.00	830207	852	144	0	0	0	0	0	0	0	0
8302	42	758	560	1	1.00	830207	934	144	0	0	0	0	0	0	0	0
8302	43	758	550	1	1.00	830207	1039	149	0	0	0	2	0	0	0	2
8302	44	758	540	1	1.00	830207	1122	149	0	0	0	2	0	0	0	2
8302	45	758	530	1	1.00	830207	12 0	151	0	0	1	1	0	0	0	2
8302	46	758	520	1	1.00	830207	1235	151	0	0	0	3	0	0	0	3
8302	47	758	510	1	1.00	830207	1316	153	0	0	0	0	0	0	0	0
8302	49	758	495	1	1.00	830207	1430	150	0	0	0	0	0	0	0	0
8302	50	767	480	1	1.00	830207	17 9	150	0	0	0	0	0	0	0	0
8302	51	767	490	1	1.00	830207	1828	151	0	0	1	0	0	0	0	1
8302	52	767	500	1	1.00	830207	2046	152	0	0	0	0	0	0	0	0
8302	53	767	510	1	1.00	830207	2225	151	0	3	1	0	0	0	0	4
8302	54	767	520	1	1.00	830208	2443	152	0	0	0	0	0	0	0	0
8302	55	767	530	1	1.00	830208	131	152	0	0	0	0	0	0	0	0
8302	56	767	540	1	1.00	830208	217	151	0	0	1	0	0	0	0	1
8302	57	767	550	1	1.00	830208	3 6	151	0	1	1	0	0	0	0	2
8302	58	767	560	1	1.00	830208	528	151	0	0	1	0	0	0	0	1
8302	59	767	570	1	1.00	830208	622	143	0	0	1	0	0	0	0	1
8302	64	775	590	1	1.00	830208	1414	145	0	0	1	0	0	0	0	1
8302	65	775	580	1	1.00	830208	1453	145	0	0	0	0	0	0	0	0
8302	66	775	570	1	1.00	830208	1529	153	0	0	0	1	0	0	0	1
8302	67	775	560	1	1.00	830208	1615	151	0	0	1	1	0	0	0	2
8302	68	775	550	1	1.00	830208	17 3	148	0	0	0	0	0	0	0	0
8302	69	775	540	1	1.00	830208	1824	148	0	0	0	0	0	0	0	0
8302	70	775	530	1	1.00	830208	2044	152	0	0	1	0	0	0	0	1
8302	71	775	520	1	1.00	830208	2121	152	0	0	0	0	0	0	0	0
8302	72	775	510	1	1.00	830208	2315	152	0	0	0	0	0	0	0	0
8302	73	775	500	1	1.00	830209	2410	151	0	2	2	0	0	0	0	4
8302	74	775	495	1	1.00	830209	150	149	0	7	1	0	0	0	0	8
8302	75	783	510	1	1.00	830209	314	151	0	0	1	0	0	0	0	1

Table 5c.—Partial listing of output data file (FORO36.DAT). Number of northern anchovy eggs and estimated ages (in hours) are grouped by developmental stages I-XI. (Cruise no. is 8302.)

Cruise no.	Tow no.	CalCOFI line	CalCOFI stn.	Stratum	Weighting factor	Date	Region	Tow time (h)	Temp. (°C)	No. eggs and ages (h)/egg stages										
										I	II	III	IV	V	VI	VII	VIII	IX	X	XI
302	19	741	530	1	1.000	830206	1	113	14	0 3.	0 4.	0 23	0 27.	0 27	0 30	0 51.	3 51	0 51	0 57	0 73
302	24	750	485	1	1.000	830206	1	619	14	0 4.	0 8	0 8	0 31	0 32	0 32	0 51	0 56	0 56	0 57	0 61
302	26	750	500	1	1.000	830206	1	1539	15	0 0.	0 11	0 17	0 17	0 19	0 41	0 41	0 41	0 41	2 62	0 65
302	27	750	510	1	1.000	830206	1	1717	15	0 0	0 3	0 19	0 19.	0 19	0 42	0 43	0 43	0 43	0 62	0 66
302	29	750	520	1	1.000	830206	1	2232	15	0 0	0 3	0 21	0 24	0 24.	0 26.	0 46	0 48	0 48	0 50	0 65
302	30	750	530	1	1.000	830206	1	2311	14	0 1	0 4	0 23	0 25	0 25.	0 46	0 49	0 49	0 49	0 69	0 73
302	31	750	540	1	1.000	830207	1	24 8	14	0 2	0 4	0 23	0 26.	0 26	0 46	0 50	0 50	0 50	0 69	0 73
302	32	750	550	1	1.000	830207	1	2449	14	0 2	0 4	0 23	0 26	0 26	0 46	0 50	0 50	0 50	2 69	0 73
302	33	750	560	1	1.000	830207	1	147	14	0 3	0 4	0 23	0 27	0 27	0 30	0 51	0 51	0 51	2 57	0 73
302	34	750	570	1	1.000	830207	1	231	14	0 4	0 4	0 7	0 28	0 28	0 30	0 51	0 52	0 52	1 57	0 73
302	38	758	600	1	1.000	830207	1	616	14	0 4	0 8	0 8	0 31	0 32	0 32	0 51	2 56	0 56	0 57	0 61
302	39	758	590	1	1.000	830207	1	720	14	0 4.	0 9	0 9	0 31.	0 33	0 33	0 39	2 57	0 57	0 57	0 61
302	40	758	580	1	1.000	830207	1	8 7	14	0 4	0 10	0 10	0 31	0 34	0 34	0 39	0 57	2 58	0 58	0 61
302	41	758	570	1	1.000	830207	1	852	14	0 4.	0 10	0 10	0 31	0 34	0 34	0 39	0 57	0 58	0 58	0 61
302	42	758	560	1	1.000	830207	1	934	14	0 4.	0 11	0 11	0 15	0 35	0 35	0 39	0 57	0 59	0 59	0 61
302	43	758	550	1	1.000	830207	1	1039	14	0 4	0 12	0 12	0 15	0 36	0 36	0 39	0 57	2 60	0 60	0 61
302	44	758	540	1	1.000	830207	1	1122	14	0 4	0 13	0 13	0 15	0 37	0 37	0 39	0 57	2 61	0 61	0 61
302	45	758	530	1	1.000	830207	1	12 0	15	0 0	0 11	0 14	0 14	0 35	1 38	0 38	0 40	0 61	1 62	0 62
302	46	758	520	1	1.000	830207	1	1235	15	0 0	0 11	0 14	0 14	0 35	0 38	0 38	0 40	2 61	1 62	0 62
302	47	758	510	1	1.000	830207	1	1316	15	0 0.	0 11	0 15	0 15	0 19	0 39	0 39	0 40	0 41	0 62	0 63
302	49	758	495	1	1.000	830207	1	1430	15	0 0.	0 11	0 16	0 16	0 19	0 40	0 40	0 40	0 41	0 62	0 64
302	50	767	480	1	1.000	830207	1	17 9	15	0 0	0 11	0 19	0 19	0 19	0 42	0 43	0 43	0 43	0 62	0 66
302	51	767	490	1	1.000	830207	1	1828	15	0 0	0 3	0 20	0 20	0 20	0 42	1 44	0 44	0 44	0 62	0 66
302	52	767	500	1	1.000	830207	1	2046	15	0 0.	0 3	0 21	0 22	0 22	0 26	0 46	0 46	0 46	0 50	0 66
302	53	767	510	1	1.000	830207	1	2225	15	0 0	0 3	0 21	3 24	0 24	0 26	0 46	1 48	0 48	0 50	0 66
302	54	767	520	1	1.000	830208	1	2443	15	0 2	0 3	0 5	0 26	0 26	0 26	0 46	0 50	0 50	0 50	0 54
302	55	767	530	1	1.000	830208	1	131	15	0 3.	0 3	0 5	0 27	0 27	0 27	0 46	0 51	0 51	0 51	0 54
302	56	767	540	1	1.000	830208	1	217	15	0 4.	0 4	0 5	0 28	1 28	0 28	0 34	0 52	0 52	0 52	0 54
302	57	767	550	1	1.000	830208	1	3 6	15	0 4.	1 5	0 5	0 28	1 29	0 29	0 34	0 52	0 53	0 53	0 54
302	58	767	560	1	1.000	830208	1	528	15	0 4.	0 7	0 7	0 28	1 31	0 31	0 34	0 52	0 55	0 55	0 55
302	59	767	570	1	1.000	830208	1	622	14	0 4.	0 8	0 8	0 31.	1 32	0 32	0 51	0 56	0 56	0 57	0 61
302	64	775	590	1	1.000	830208	1	1414	14	0 0.	0 12	0 16	0 16.	1 38	0 40	0 40	0 45	0 64	0 64	0 64
302	65	775	580	1	1.000	830208	1	1453	14	0 0.	0 12	0 16	0 16	0 38	0 40	0 40	0 45	0 64	0 64	0 64
302	66	775	570	1	1.000	830208	1	1529	15	0 0.	0 11	0 17	0 17	0 19	0 41	0 41	0 41	0 41	1 62	0 65
302	67	775	560	1	1.000	830208	1	1615	15	0 0	0 11	0 18	0 18	0 19	0 42	1 42	0 42	0 42	0 62	1 66
302	68	775	550	1	1.000	830208	1	17 3	14	0 0.	0 12	0 19	0 19	0 22	0 43	0 43	0 45	0 67	0 67	0 67
302	69	775	540	1	1.000	830208	1	1824	14	0 0	0 12	0 20	0 20	0 22	0 44	0 44	0 45	0 67	0 68	0 68
302	70	775	530	1	1.000	830208	1	2044	15	0 0.	0 3	0 21	0 22	0 22	0 42	0 46	1 48	0 46	0 50	0 66
302	71	775	520	1	1.000	830208	1	2121	15	0 0.	0 3	0 21	0 23	0 23	0 26	0 46	0 47	0 47	0 50	0 66
302	72	775	510	1	1.000	830208	1	2315	15	0 1	0 3	0 21	0 25	0 25	0 26	0 46	0 49	0 49	0 50	0 54
302	73	775	500	1	1.000	830209	1	2410	15	0 2	0 3	0 5	2 26	0 26	0 26	0 46	0 50	2 50	0 50	0 54
302	74	775	495	1	1.000	830209	1	150	14	0 3	0 4	0 23	0 27	5 27	0 30	0 51	0 51	1 51	0 57	0 73
302	75	783	510	1	1.000	830209	1	314	15	0 4	0 5	0 5	0 28	0 29	0 29	1 34	0 52	0 53	0 53	0 54

Table 5d.—Partial listing of the output data file (FORO37.DAT): estimated age (h) and number of northern anchovy eggs for those egg stages observed within or beyond the normal ranges of expected tow time ($\hat{T}\pm G$, see text). (Cruise no. is 8302.)

Cruise no.	Tow no.	CalCOFI line	CalCOFI stn.	Stratum	Weighting factor	Date	Region	Tow time (h)	Temp. (°C)	Est. age (h)	Lower limit of age	Upper limit of age	Egg stage	No. of eggs	Expected tow time (h)
302	26	750	500	1	1.000	830206	1	15	39	15	62	50	62	10	2.	6.
302	32	750	550	1	1.000	830207	1	24	49	14	69	57	69	10	2.	13.
302	33	750	560	1	1.000	830207	1	1	47	14	57	57	69	10	2.	13.
302	34	750	570	1	1.000	830207	1	2	31	14	57	57	69	10	1.	13.
302	39	758	590	1	1.000	830207	1	7	20	14	57	45	57	8	2.	2.
302	45	758	530	1	1.000	830207	1	12	0	15	62	50	62	10	1.	6.
302	46	758	520	1	1.000	830207	1	12	35	15	61	41	61	9	2.	1.
302	46	758	520	1	1.000	830207	1	12	35	15	62	50	62	10	1.	6.
302	64	775	590	1	1.000	830208	1	14	14	14	38	22	38	5	1.	4.
302	66	775	570	1	1.000	830208	1	15	29	15	62	50	62	10	1.	6.
302	67	775	560	1	1.000	830208	1	16	15	15	66	54	66	11	1.	10.
302	74	775	495	1	1.000	830209	1	1	50	14	4	4	12	2	2.	6.
302	75	783	510	1	1.000	830209	1	3	14	15	34	34	46	7	1.	14.
302	76	783	520	1	1.000	830209	1	4	5	15	34	34	46	7	1.	14.
302	86	791	590	1	1.000	830209	1	12	43	15	62	50	62	10	2.	6.
302	92	791	530	1	1.000	830209	1	16	45	15	42	26	42	6	3.	8.
302	92	791	530	1	1.000	830209	1	16	45	15	62	50	62	10	3.	6.
302	98	800	540	1	1.000	830210	1	4	12	15	4	0	4	1	3.	24.
302	98	800	540	1	1.000	830210	1	4	12	15	52	40	52	8	2.	20.
302	100	800	560	1	1.000	830210	1	9	0	15	11	3	11	2	3.	5.
302	100	800	560	1	1.000	830210	1	9	0	15	35	19	35	5	2.	1.
302	102	800	580	1	1.000	830210	1	11	30	15	11	3	11	2	1.	5.
302	108	808	570	1	1.000	830210	1	19	53	15	66	54	66	11	1.	10.
302	133	817	580	1	1.000	830214	1	2	33	15	4	0	4	1	10.	24.
302	133	817	580	1	1.000	830214	1	2	33	15	28	12	28	4	3.	18.
302	133	817	580	1	1.000	830214	1	2	33	15	34	34	46	7	1.	14.
302	133	817	580	1	1.000	830214	1	2	33	15	52	40	52	8	1.	20.
302	134	817	590	1	1.000	830214	1	3	10	15	4	0	4	1	2.	24.
302	139	825	570	1	1.000	830214	1	7	20	15	4	0	4	1	1.	24.
302	146	825	490	1	1.000	830214	1	12	32	15	61	41	61	9	1.	1.
302	155	833	406	1	1.000	830214	2	19	36	15	0	0	4	1	10.	24.
302	155	833	406	1	1.000	830214	2	19	36	15	3	3	11	2	4.	5.
302	165	841	450	1	1.000	830215	2	9	20	16	10	2	10	2	11.	4.
302	165	841	450	1	1.000	830215	2	9	20	16	56	44	56	10	5.	24.
302	165	841	450	1	1.000	830215	2	9	20	16	59	47	59	11	3.	3.
302	166	841	440	1	1.000	830215	2	10	5	16	59	47	59	11	3.	3.
302	172	850	410	1	1.000	830215	2	16	0	16	42	30	42	7	2.	10.
302	173	850	420	1	1.000	830215	2	16	35	16	42	30	42	7	4.	10.
302	178	850	470	1	1.000	830215	2	20	54	15	0	0	4	1	1.	24.
302	178	850	470	1	1.000	830215	2	20	54	15	3	3	11	2	1.	5.
302	178	850	470	1	1.000	830215	2	20	54	15	66	54	66	11	2.	10.
302	179	850	480	1	1.000	830215	2	22	20	15	0	0	4	1	2.	24.

Table 5e.—Partial listing of data file (FORO38.DAT): Number of eggs and age (in days) excluding eggs >2.5 d old. (Cruise no. is 8302.)

Cruise no.	Tow no.	CalCOFI line	CalCOFI stn.	Date	Number of eggs	Age (in days)
302	6	0733	0550	830205	0.0000	0.4583
302	6	0733	0550	830205	0.0000	1.5694
302	6	0733	0550	830205	1.0000	2.5417
302	17	0741	0550	830205	1.0000	1.0417
302	17	0741	0550	830205	0.0000	2.0104
302	17	0741	0550	830205	0.0000	2.9583
302	18	0741	0540	830206	0.0000	0.8229
302	18	0741	0540	830206	1.0000	2.0833
302	18	0741	0540	830206	0.0000	2.9583
302	19	0741	0530	830206	0.0000	0.8438
302	19	0741	0530	830206	3.0000	2.1250
302	19	0741	0530	830206	0.0000	2.7083
302	24	0750	0485	830206	0.0000	0.2778
302	24	0750	0485	830206	0.0000	1.5208
302	24	0750	0485	830206	0.0000	2.3958
302	26	0750	0500	830206	0.0000	0.6667
302	26	0750	0500	830206	0.0000	1.7083
302	26	0750	0500	830206	2.0000	2.5833
302	27	0750	0510	830206	0.0000	0.7917
302	27	0750	0510	830206	0.0000	1.7813
302	27	0750	0510	830206	0.0000	2.6667
302	29	0750	0520	830206	0.0000	0.9896
302	29	0750	0520	830206	0.0000	2.0000
302	29	0750	0520	830206	0.0000	2.7500
302	30	0750	0530	830206	0.0000	0.8021
302	30	0750	0530	830206	0.0000	2.0104
302	30	0750	0530	830206	0.0000	2.9583
302	31	0750	0540	830207	0.0000	0.8229
302	31	0750	0540	830207	2.0000	2.0833
302	31	0750	0540	830207	0.0000	2.9583
302	32	0750	0550	830207	0.0000	0.8229
302	32	0750	0550	830207	0.0000	2.0417
302	32	0750	0550	830207	2.0000	2.8750
302	33	0750	0560	830207	0.0000	0.8438
302	33	0750	0560	830207	0.0000	1.9063
302	33	0750	0560	830207	2.0000	2.3750
302	34	0750	0570	830207	0.0000	0.2083
302	34	0750	0570	830207	0.0000	1.4271
302	34	0750	0570	830207	1.0000	2.3750
302	38	0758	0600	830207	0.0000	0.2778
302	38	0758	0600	830207	0.0000	1.5208
302	38	0758	0600	830207	2.0000	2.3333
302	39	0758	0590	830207	0.0000	0.3056
302	39	0758	0590	830207	0.0000	1.4167
302	39	0758	0590	830207	2.0000	2.3750
302	40	0758	0580	830207	0.0000	0.3333
302	40	0758	0580	830207	0.0000	1.4375

Table 6.—Number of northern anchovy eggs per tow grouped by days S(0-3 h), A (4-27 h), B (28-51 h), C (52-75 h), and D (76-99 h) from the manual system (m) and the proposed automated system (a) for each year in 1980-83.

		No. eggs/day categories					Disintegrated		No.
		S[1]	A	B	C	D	eggs	Total[2]	tows[3]
1980	m	2.72	13.65	8.86	4.91	0.16	0.82	31.14	312
	a	2.61	13.74	9.78	4.08	0.03		30.24	
1981	m	2.76	12.32	10.33	6.91	0.47	0.85	33.72	564
	a	1.81	13.86	12.00	4.98	0.13		32.78	
1982	m	2.49	9.00	7.00	4.44	0.31	0.89	24.23	308
	a	1.13	10.50	7.77	3.78	0.16		23.34	
1983	m	1.95	9.16	8.42	2.88	0.002	0.67	23.07	482
	a	2.01	9.31	8.51	2.61			22.44	

[1]S eggs from manual system (m) include some disintegrated eggs.
[2]Difference between "m" and "a" is the disintegrated eggs which were included in "m," but not in "a."
[3]Positive tows only.

DISCUSSION

The automated procedure should be fully tested before being implemented. For the anchovy egg data, 1980-83 data of aged eggs from both manual and automated procedures were compared based upon positive tows (tows with at least one egg sampled) (Table 6). Although there were some differences between the results of these two procedures, the results from the automated procedure are encouraging. The differences are due to different methods of age assignment and to subjective elements in the manual age assignment method. In the manual system, day category (S, A, B, C, D, and E) was first assigned to eggs (Stauffer and Picquelle 1985). An actual age was then computed based upon the time of tow (k) and day category:

$$\text{age} = k+2+24\times f \qquad (8)$$

where $f = 0,1,2,\ldots$ for A,B,C,. . .-day eggs. In the automated system, the age was assigned according to Equation (7) before the day category was assigned. Equation (8) yields a range of ages larger than that from Equation (7) and thus gives larger values for maximum ages. This difference affects the ages of older eggs, particularly in the case where eggs older than 2.5 d are excluded in computation of egg mortality. For example, at temperature 15°C, the maximum age for stage XI is 66 h using Equation (7), and the maximum age is 72 h using Equation (8) for eggs that were sampled at 0100. Thus, more eggs would be assigned to older age groups when Equation (8) was used rather than Equation (7). The subjectivities of human judgment or human errors of the manual system are evident in several steps in the manual methods. These include 1) misassignment of age from the key structure due to inexperience or the fatigue of the worker; 2) determination of age for eggs which were observed at a time significantly different from the expected time (Table 2); and 3) assignment of age to eggs observed around 0200 which is the break point of A, B, C, D, and E-day categories (Fig. 2).

To adopt the program for other species would require 1) a model for the temperature-dependent egg development, e.g., Equation (4); 2) the distribution of age within each egg stage to obtain G in Equation (6); and 3) the peak spawning time (st) for Equation (5), so that a reference table can be constructed which is the base of the automated procedure.

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A Protocol for Designing a Sea Survey for Anchovy Biomass Assessment

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ABSTRACT

The objective of obtaining a representative sample of the adult northern anchovy population, for the purposes of determining reproductive status, is discussed. Several considerations affecting this objective are listed, including deterioration of tissue, vulnerability to sampling gear, geographic variability in reproductive behavior, logistic limitations, and secondary indicators of the presence of fish. The timing and duration of a survey, the laboratory equipment required, and potential problems are discussed. Specific specifications for a desired survey vessel are also outlined including sea day capability, gear operations, vessel speed, personnel, storage, work space, and cruise plan.

SEA SURVEY DESIGN AND METHODS

Collection Gear Requirements

The primary requirement for trawl collection gear is that it provides a representative sample of the adult anchovy population. Because it is the average fish that is of interest, rather than the average location, sampling density should parallel the density of fish. Thus, relatively fewer samples need be taken in sparsely populated waters near the edge of the range; however, these areas must be covered sufficiently well to confirm the extent of the range. Adult sampling should coincide as much as possible with the time and area of egg samples. Several considerations make this requirement difficult to achieve. These considerations include:

1) Fish must be preserved in a fresh condition so that histological criteria may be used to determine their reproductive status. The Southwest Fisheries Center currently requires that tissue be fixed within an hour of being caught, and this appears to be adequate. (Adult surveys conducted with a purse seiner off Peru extended this period to 2 h without adverse consequences; J. Alheit[2].) Because it is not possible to determine when a fish was captured, trawls must be of short duration so as to set and retrieve the net, subsample the catch, expose the gonads, and preserve the fish within the specified time period. The same must be required of alternative sampling gears such as purse seine or lampara nets.

2) Some portions of the adult population may be more vulnerable to capture than others; catches may vary with sampling gears, with time of day, and with reproductive status. In 1981, actively spawning female *Engraulis mordax* were oversampled with a trawl relative to females which had spawned the day before; purse seine catches, paired with the trawls, yielded equal proportions of active and postactive spawners. The sex ratio, determined from the trawl sample, was biased (towards males) only during the hours of spawning whereas the purse seine selectively caught females regardless of the time of day. The 1982 trawl survey caught equal proportions of active and postactive spawners although the sex ratio changed during the hours of spawning favoring the males (Picquelle and Hewitt, 1983). In contrast, a 1981 purse seine sample of Peruvian anchovy, *Engraulis ringens*, obtained off Peru oversampled actively spawning females as well as showed a diurnal pattern in the sex ratio of caught fish (Alheit et al., 1984). Furthermore, a comparison of the distribution of actively spawning females with a binomial distribution suggested that the fish were not independently distributed in the collections; fish which had spawned the day before appeared to be independently distributed. It was concluded from these observations that males and actively spawning females segregate out from other females during the peak hours of spawning at a depth (or an area) where they are more vulnerable to the trawl (Alheit et al., 1984). This behavior appears to begin early in the day before the ovaries hydrate and ends a few hours after spawning.

Bias due to gear selectivity, temporal or geographic distribution, or fish behavior should be watched for. Because it is not always evident, every set of adult collections should be checked for possible bias. Sampling gears and procedures should be modified so as to minimize potential bias. The ideal sampler is one that is invisible to the fish, to preclude avoidance, and one that samples through the entire vertical range of their distribution. If the source of bias cannot be eliminated, it may be corrected by use of appropriate availability factors and/or statistical stratification. If size of fish

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follows an oceanographic or geographic pattern, the samples must cover the range. For fishes which spawn very locally in time or space, representative sampling may be difficult, while for widely distributed spawners representative sampling may not be difficult to achieve. 3) Spawning may occur in areas where it is difficult to sample the adults. These include the nearshore and other shallow water zones where there is not sufficient depth to fish a trawl or set a purse seine net. *Engraulis mordax* spawns in the nearshore zone with the same intensity as observed throughout its habitat (Hewitt and Brewer, 1983); however, the reproductive output of adults in this zone must be inferred because of the difficulty in sampling there. It may be necessary to choose a sampling gear, which can be deployed in shallow or obstructed areas (such as a lampara net), for those populations that deposit a large portion of their spawn in shallow waters. 4) Time and personnel requirements must also be considered when selecting a sampling gear. As mentioned above, a gear should be eliminated if it cannot be set, retrieved, and the catch preserved in less than a specified time period. The vessels available and their speed of travel between stations may be determined by the choice of sampling gear. In general, a purse seine requires more people to deploy than does a trawl; however, there may be considerable variation between boats. One to two people are required to subsample and preserve the catch in addition to the personnel necessary to fish the gear.

Secondary information may be useful for improving sampling efficiency, but care must be taken not to cause unrepresentative sampling. For example, presence of eggs in the plankton tows indicates presence of fish, but absence of eggs does not imply absence of fish. Adult samples from areas of low spawning rates may be very important in obtaining a good biomass estimate. Secondary information may improve trawl success rates. Such information may include signs of fish encountered during recent survey operations, signs of predators, and results of other observers such as the fishery, airplane pilots, or other concurrent research cruises. If fish behavior is known, oceanographic information on water temperature, clarity, etc., may provide useful clues to presence and absence of fish.

It is highly recommended that adult collections be examined for evidence of avoidance and selectivity. In this regard, it is a good practice to sample the catch for age composition. This information may be used as additional criteria to compare gears, geographic areas, and time of day.

SURVEY TIMING AND DURATION

The timing of the survey should be based on biological information. The survey should be conducted during peak spawning activity of the adults. The number of samples required to estimate the spawning fraction increases exponentially as that fraction decreases (P. E. Smith[3]) so that the additional cost of sampling outside the spawning season rapidly becomes unacceptable. It is absolutely necessary that adult sampling be conducted simultaneously with egg sampling, either on the same vessel or on different vessels working in the same vicinity.

The duration of the survey depends on logistical considerations. These include area of the survey, desired sampling intensity, distance between stations and speed of the vessel, and fraction of the day available to conduct adult sampling. If more than one vessel is available, sampling may be conducted 24 hours/day, which would allow more stations to be occupied in the same amount of time. However, the fish may be less catchable during daylight hours, either because they are deeper or because they can better avoid the net. As with the choice of gear, a compromise must be made between quantity and quality of the data (i.e., precision and accuracy).

EQUIPMENT AND SUPPLIES

Adult sampling equipment can be divided into two general categories: Sampling gear and laboratory supplies. Sampling gear is usually specific to the vessel employed for the survey and is discussed below under "Vessel Requirements." Laboratory equipment refers to everything necessary to subsample the catch and preserve the specimens: buckets for subsampling, preserving solution, labels, data sheets, and specimen jars. Also useful are a small scoop, specimen jar rack, dissecting scissors, small spatula, forceps, and a hand digital logger with at least three registers (e.g., blood cell counter). The survey should be planned in enough detail to take the proper amount of supplies. Because space on a vessel is usually at a premium and because running short of supplies is also costly, it is recommended that extra supplies be kept onboard.

SOURCES OF ERRORS, BIASES, AND POTENTIAL PROBLEMS

The most serious source of bias is the selectivity of adult fish sampling gear. As discussed above, suspected biases should be investigated and, if necessary, changes should be made to the gear and/or sampling procedures. A representative sample is achieved when each adult fish has an equal probability of being caught by the sampler; if ripe females are more vulnerable to capture than the rest of the adult population or if fish were more able to avoid the net during certain times of the day, then a representative sample is not achieved, and this fact must be considered. In the survey for *Engraulis mordax*, where these problems were encountered, ripe females were eliminated from the calculation of the spawning fraction and adult sampling was conducted during only a portion of the day.

Error may be introduced when there is geographic heterogeneity in spawning and homogeneous sampling. Close examination of the results and postsurvey stratification, if indicated, is the most direct method of reducing this error. Error may also be introduced when subsampling the catch aboard ship (e.g., an inadvertent preference for large fish); this may be reduced by devising a systematic procedure for selecting fish. Finally, it is important to clean the net between sets; fish can get caught in the webbing and become part of the apparent catch of a subsequent set. Off California, pelagic red crabs sometimes contaminate the catch and abrade the fish; these fish cannot be distinguished from the residual of a previous trawl unless the net is thoroughly flushed between sets.

VESSEL REQUIREMENTS

Because the survey vessel is the most important piece of equipment to be specified, it is prudent to consider the requirements in some detail. The following subsections outline the minimum considerations. Additional specifications and detail in planning will aid in the conduct of the survey. It is impossible to prepare too much.

[3]P. E. Smith, Southw. Fish. Cent., Natl. Mar. Fish. Serv., NOAA, La Jolla, CA 92038, pers. commun. Sept. 1983.

Sea Days Capability

The vessel must be able to take on sufficient provisions and fuel to conduct a major portion of the survey without returning to port. A minimum two-week capability should be required to maintain survey continuity and to obtain samples in a reasonably short period of time. Bear in mind that a port call involves travel time to port, time in port, and travel time back to the next station; delays in sampling can easily become longer than originally anticipated.

Gear Operations

The vessel should be equipped with the specified adult sampling gear rather than bringing new gear aboard. The gear should be clean and in good operating order. The vessel's crew should be proficient in deploying and retrieving the gear as well as repairing minor damage to the net without returning to port. If members of the scientific party will be required to help operate the gear, the number of people and their duties should be clearly specified.

The winch requirements (as outlined above for egg sampling) should be clearly specified and stressed as critical to the conduct of the survey. Other requirements include provisions for measuring the wire angle and washing down the net.

It is most important that any collection operations (egg or adult) which involve the cooperation of the crew and the scientific party be discussed thoroughly and even practiced before the survey begins.

Vessel Speed

Knowledge of the vessel's speed is a key element in the planning of a survey because a major portion of time is spent underway between stations. Speed will vary according to sea conditions and the loading of the vessel; however, a realistic estimate of the expected average speed is very useful. The survey may be planned first and a minimum vessel speed specified as a criterion for vessel selection.

Personnel

The primary requirement for the vessel personnel is that they be adequate in number and sufficiently skilled to accomplish the specified operations. Competence in navigation should be a major requirement; this not only involves human skill but the proper equipment as well. It should be specified that each station must be accurately located and that it is not sufficient to know only the vessel position relative to port or land. As noted above, vessel personnel should know how to operate and repair the adult sampling gear.

Supplies and Specimen Storage

The vessel must have adequate space dedicated to dry storage of sampling supplies and specimens. These requirements must be calculated from the survey plan and can be considerable, particularly for the adult fish specimens. In addition to dry storage, storage space on deck may be specified for sampling gear and freezer storage may be necessary for some specimens.

Laboratory and Work Space

The vessel must have a covered space, well lit and well ventilated, that can be dedicated for use in processing specimens; running water and a drain are essential. Sufficient work space must exist on deck to store and deploy the egg sampler and the adult sampler, to remove their catches, and to subsample in the case of adult catches.

CRUISE PLAN AND WORK SCHEDULES

A cruise plan, distributed to all personnel involved (vessel crews and scientific parties) greatly increases the chance for a successful survey. Such a plan should include a brief statement of the cruise objectives, schedule of port calls, map of the stations and the order in which they are to be occupied, activities to be accomplished at each station, list of the personnel participating and those in charge, and a list of the equipment necessary to accomplish the objectives.

Work schedules should also be made and distributed prior to the cruise. These schedules alert participants as to when they will be working and what they will be doing so that they may be prepared. Careful thought should be given to how many people will be required on each vessel and whether the vessel can accommodate them. As an example, conducting operations 24 hours a day will require two or three work shifts for egg sampling, plus additional people for adult sampling. Each shift should have a designated leader, and one person, either from the crew or from the scientific party, should be in charge of adult sampling operations.

The value of a good plan cannot be emphasized enough. The plan should convey to all participants a clear, concise statement of what is to be accomplished on the cruise and how it is to be done. It should give the impression that a considerable amount of thought and preparation has already been done, and what remains is to carry out the plan. A survey cruise rapidly becomes tedious as the same operations are performed over and over; the plan helps people mark time and keep track of progress.

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Sampling Requirements for the Adult Fish Survey

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ABSTRACT

The question of how many fish should be collected to estimate population fecundity parameters is addressed. The goal is to determine the optimal combination of the number of stations to occupy (n) and the number of fish to subsample per station (m) that will provide the minimum variance of parameter estimates. The collection of mature fish for this purpose is considered to be a two-stage sample design in which the total number of fish processed is equal to n times m. In most cases, the subsample of fish taken from a trawl station can be treated as a cluster sample, and a simple relationship between variance of the parameters and the sample sizes, n and m, may be established. Spawning fraction, the parameter with the largest relative variance, is chosen to evaluate alternative combinations of n and m. In terms of the total number of fish processed, it is generally more efficient in a statistical sense to occupy more trawl stations and to subsample fewer fish per station than vice-a-versa. If the major cost of collecting data is associated with ship operations, then it is cheaper to occupy fewer trawl stations and subsample more fish per station. The equations and Figure 1, based on northern anchovy data from California, provide criteria for determining the combination of n and m which achieves desired levels of variance.

INTRODUCTION

The number of independent observations directly impacts the estimated variance of a parameter; the higher the number of observations, the smaller the variance. A trawl survey is usually a two-stage sample design; hence the number of observations is determined by the number of trawls taken (n) and the number of fish subsampled from each trawl (m). However, for a fixed total sample size (nm), varying combinations of n and m will produce varying values of the estimated variance because the fish within a trawl are typically more similar than fish between trawls, that is, fish within trawls are positively correlated and hence are not independent observations. For example, the intratrawl correlation coefficient for female weight data collected during the 1980 survey was 0.60. Thus it is advantageous to find the optimal combination of n and m that will produce the minimum variance.

SAMPLE SIZE RELATIONSHIPS

In two-stage sampling, the estimates of the population mean and variance are (Cochran 1963),

$$\bar{\bar{x}} = \sum_{i=1}^{n} \frac{\bar{x}_i}{n} \quad \text{where } \bar{x}_i = \sum_{j=1}^{m} \frac{x_{ij}}{m} \tag{1}$$

$$\text{and } \hat{var}(\bar{\bar{x}}) = (1 - f_1)\frac{s_1^2}{n} + f_1(1 - f_2)\frac{s_2^2}{nm} \tag{2}$$

where $s_1^2 = \dfrac{\sum_{i=1}^{n} (\bar{x}_i - \bar{\bar{x}})^2}{n - 1}$ = intertrawl component of variance,

$s_2^2 = \dfrac{\sum_{i=1}^{n}\sum_{j=1}^{m} (x_{ij} - \bar{x}_i)^2}{n\,(m - 1)}$ = intratrawl component of variance,

$f_1 = n/N$, where N is the total number of stations, and
$f_2 = m/M$, where M is the total number of elements at each station.

(Note: for simplicity's sake, two-stage sampling with stations of equal size is used here for illustration purposes.) Equation (2) relates variance to the values of n and m, and this equation can be used to find the optimal values of n and m which will produce the minimum variance.

But first, some simplifications can be made. In the majority of fisheries surveys, the sampling fraction f_1 is negligibly small so that the value $f_1 = 0$ may be substituted into equation (2). This reduces to

$$\hat{var}(\bar{\bar{x}}) = \frac{s_1^2}{n} \tag{3}$$

and the intratrawl component of variance disappears. Because of this simplification, the trawl sample may be treated like a cluster sample. In cluster sampling, the trawl average, $\bar{x}_1$, is measured without error, i.e., every fish is measured. In two-stage sampling, the trawl average is estimated with error, but in this case that error is negligible and does not impact the total variance estimate.

By considering the subsample of fish taken from a trawl as a cluster sample, a simpler relationship between variance and values of n and m may be established. The intracluster correlation (ρ) may be expressed as a function of the ratio of the cluster-sample variance to the random-sample variance:

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$$\frac{\sigma^2_{\bar{\bar{x}}\,cluster}}{\sigma^2_{\bar{\bar{x}}\,random}} = 1 + \rho\,(m-1) \tag{4}$$

where
$$\sigma^2_{\bar{\bar{x}}\,cluster} = \frac{\sum_{i=1}^{n}(\bar{x}_i - \bar{\bar{x}})^2}{n\,(n-1)} \tag{5}$$

and
$$\sigma^2_{\bar{\bar{x}}\,random} = \frac{\sum_{i=1}^{n}\sum_{j=1}^{m}(x_{ij} - \bar{\bar{x}})^2}{nm\,(nm-1)}\,. \tag{6}$$

The fish sampled during the trawl survey are used to estimate several parameters, i.e., sex ratio, spawning fraction, fecundity, and female weight. The optimal combination of m and n will not be the same for all parameters. Hence, the parameter with the largest relative variance is chosen to pick the values of m and n. This parameter is spawning fraction for the northern anchovy example.

Spawning fraction is distributed approximately as a binomial distribution. We can simplify equation (4) by substituting the variance of a binomial distribution for the random-sample variance

$$\sigma^2_{\bar{\bar{x}}\,random} = \sigma^2_{\bar{\bar{x}}\,binomal} = \frac{\bar{\bar{x}}\,(1-\bar{\bar{x}})}{nm}\,. \tag{7}$$

Solving equation (4) for m after substituting equation (7) for $\sigma^2_{\bar{\bar{x}}\,random}$ gives

$$m = \frac{\bar{\bar{x}}\,(1-\bar{\bar{x}})(1-\rho)}{n\sigma^2_{\bar{\bar{x}}\,cluster} - \bar{\bar{x}}\,(1-\bar{\bar{x}})\rho}\,. \tag{8}$$

Values for $\bar{\bar{x}}$, ρ and $\sigma^2_{\bar{\bar{x}}\,cluster}$ are needed to specify the relationship between m and n. It is more convenient to work with the coefficient of variation (cv) rather than variance directly because one can then specify the desired precision in terms of percent of the parameter rather than in absolute terms. This changes equation (8) to

$$m = \frac{(1-\bar{\bar{x}})(1-\rho)}{n\bar{\bar{x}}\,cv^2 - (1-\bar{\bar{x}})\rho} \tag{9}$$

where
$$cv = \frac{\sigma_{\bar{\bar{x}}\,cluster}}{\bar{\bar{x}}}\,.$$

Considering a range of values for the coefficient of variation is useful to see how n and m change as the precision of the estimate of $\bar{\bar{x}}$ changes. It is also worthwhile to consider a range of values for $\bar{\bar{x}}$, because estimates of this are not available until after the survey, but it is possible to specify a range of values within which $\bar{\bar{x}}$ is likely to fall. A value for ρ may be selected using an estimate from previous surveys.

NUMERICAL EXAMPLE

As an example, the attached series of graphs (Fig. 1) was constructed using the estimate of ρ calculated from the 1982 survey data using Equation (4) ($\rho = 0.0448$). Values of $\bar{\bar{x}}$ range from 0.06 to 0.12 in increments of 0.02, with one graph corresponding to each value of $\bar{\bar{x}}$. On each graph are five lines corresponding to five values of the desired coefficient of variation (0.100 to 0.200). The vertical axis is m, the subsample size; and the horizontal axis is n, the number of trawls.

These graphs do not serve to pinpoint the precise combination of m and n that is optimal. Instead, they illustrate how n and m together determine the precision of the estimate, given the value of the estimate. They suggest general sampling strategies rather than specific criteria.

It is generally more efficient (in terms of number of fish processed) to take more trawls and fewer fish per trawl than vice-a-versa. For example, consider a spawning fraction of 0.10 and a desired coefficient of variation of 0.125. If 80 trawls are taken, then 10 fish should be subsampled, for a total of 800 fish. On the contrary, if only 50 trawls are taken, then 26 fish need to be subsampled, for a total of 1,250 fish. This is true because the fish within each trawl tend to be positively correlated and hence are not independent observations, while the fish between trawls are uncorrelated, thus contributing more information per fish.

COST CONSIDERATIONS

The major cost of data collection is associated with ship operations, not with processing the fish in the laboratory. For a fixed number of fish sampled, it is much cheaper to take fewer trawls and larger subsamples, although this reduces the precision of the estimates. Thus, what is more efficient statistically (many trawls and small subsamples) is the exact opposite of what is more efficient financially (few trawls and large subsamples). If the costs of taking a trawl (C_1) and of processing a fish (C_2) are known, these costs may be incorporated in the relationship between n and m and a new function may be derived which minimizes total cost (C),

$$C = nC_1 + nmC_2\,. \tag{10}$$

We wish to minimize C under the constraint of Equation (9).

$$C = nC_1 + nC_2\left[\frac{(1-\bar{\bar{x}})(1-\rho)}{n\bar{\bar{x}}\,cv^2 - (1-\bar{\bar{x}})\rho}\right]. \tag{11}$$

C is minimized by setting $\dfrac{\partial C}{\partial n} = 0$.

This results in the following values for n and m:

$$n = \frac{C_1(1-\bar{\bar{x}})\rho + (1-\bar{\bar{x}})\sqrt{C_1C_2\rho(1-\rho)}}{C_1\bar{\bar{x}}\,cv^2} \tag{12}$$

$$m = \left[\frac{(1-\rho)C_1}{\rho C_2}\right]^{1/2}. \tag{13}$$

Consider the example presented earlier where $\bar{\bar{x}} = 0.10$ and the desired coefficient of variation is 0.125 (again using $\rho = 0.0448$ from the 1982 survey data). Suppose the cost of taking one trawl (C_1) is \$1,000 and the cost of processing one fish (C_2) is \$25. These conditions result in the values minimizing cost of $n = 45$ and $m = 29$.

Frequently the number of trawls taken is determined by factors not related to desired precision and not directly controllable, such as the number of days at sea, weather condition, and the success rate of catching the target species. Before a survey begins, only the number of days at sea is known; however, predictions about the other two factors mentioned are available, so that an approximate number of trawls can be deduced. Then the graphs may be used to find the necessary value of m to attain the desired coefficient of variation.

Another consideration of selecting n and m is the expected number of spawning females subsampled in a trawl. If the spawning frac-

tion is 0.10, and only 10 females are subsampled from each trawl, then the expected number of spawning females sampled from each trawl is only 1, based on a binomial distribution where $n = 10$ and $p = 0.10$. This will lead to a high proportion (35% on average) of trawl subsamples with no spawning females, thus inflating the variance. By raising m to 15 females, the average percent of trawl subsamples with no spawning females drops to 21%; for 20 females the percent is 12%.

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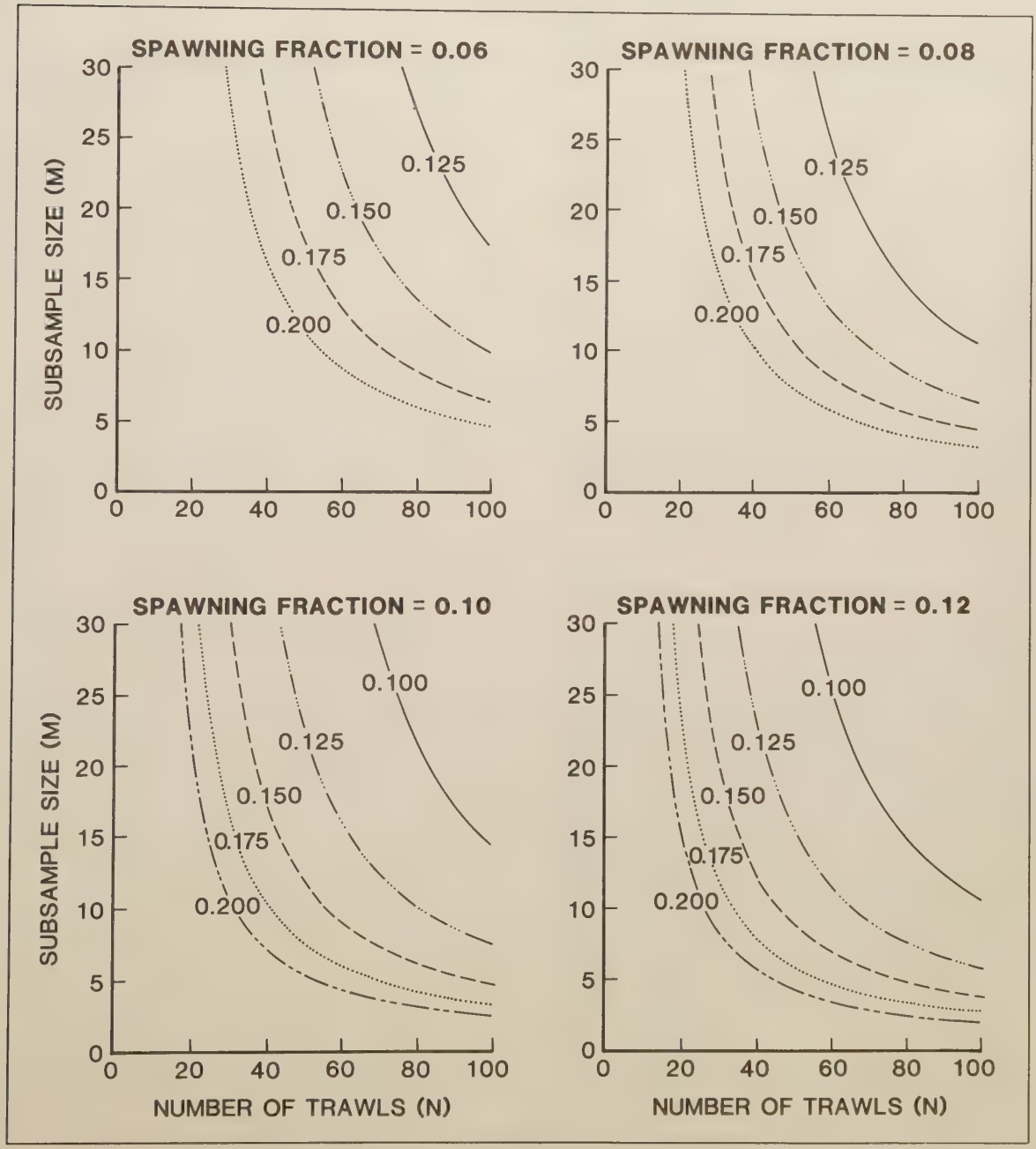

Figure 1.—Relationship between subsample size and number of trawls for a range of values for spawning fraction and coefficient of variation.

Spawning Frequency of Peruvian Anchovies Taken with a Purse Seine

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ABSTRACT

The incidence of postovulatory follicles was used to determine spawning frequency of the Peruvian anchovy, *Engraulis ringens*. Because the fish were collected with a purse seiner, samples could be obtained day and night. This allowed two independent estimates of spawning frequency, one for females taken one day after spawning and another for females taken two days after spawning. The correlation between these two estimates was tested with a paired *t*-test, and the reliability of using incidence of postovulatory follicles to determine spawning frequency was thus proven. The vulnerability of female anchovy to the purse seine changed according to their reproductive state. Females in the prespawning hydrated stage are oversampled by the purse seine and therefore cannot be used to determine spawning frequency.

INTRODUCTION

For the Southwest Fisheries Center (SWFC) egg production method, accurate estimates of percent spawning frequency by females is an essential statistic. With a representative sample, one should be able to derive three independent estimates of spawning frequency (i.e., fraction of mature females spawning per day): 1) percentage of females with hydrated oocytes; 2) percentage of females with day-1 postovulatory follicles (age: 9-32 h); and 3) percentage of females with day-2 postovulatory follicles (age: 33-56 h).

Using Hunter and Goldberg's (1980) technique of recording the incidence of postovulatory follicles, spawning frequency was determined for the Peruvian anchovy, *Engraulis ringens* (Alheit et al. 1984; Santander et al. 1984). However, an important difference in sampling techniques between California and Peru was the type of gear used to obtain females. In the California surveys, a small stern trawler was used which permitted only night catches of adult anchovies (Hunter and Goldberg 1980; Hunter and Macewicz 1980; Stauffer and Picquelle 1981). In Peru, however, anchovies were collected with a purse seiner and could therefore be sampled both night and day (Alheit et al. 1984).

Females with postovulatory follicles and females in the hydrated, prespawning stage were obtained for the entire 24-h cycle. There is possible bias in such a 24-hour sampling scheme in determination of spawning frequency introduced by the time of capture and affecting the incidence of females with postovulatory follicles (see Stauffer and Picquelle 1985). Thus, the use of a purse seine for Peruvian anchovy gave new insights into this bias problem. As hydrated females were sampled during the day with purse seine, daytime incidence of spawning frequency could be compared with night samples. The importance of night and additional day samples of anchovies for the validation of Hunter and Goldberg's (1980) method is described in this section, and the influence of different sampling gear (trawl versus purse seine) and of the reproductive behavior of mature anchovies on the obtained data of spawning frequency and sex ratio is also discussed.

RESULTS

If the spawning frequencies for day-1 and day-2 females are shown to come from populations with the same mean, their combination would double the sample size available for the determination of spawning frequency and reduce the variance of the estimate.

In order to see if the frequencies could be combined, a test of the differences between paired samples, a paired *t*-test (Snedecor and Cochran 1967), was carried out. The paired samples were the frequency of day-1 and day-2 females in each of 49 collections of 20 females each. A paired test was used due to the likely correlation between samples taken from the same collection. The null hypothesis was that the mean difference between samples was zero. The results of the test gave a t value of 1.196 which was not significant when compared to the critical value of a t distribution with $\alpha = 0.05$ and $n-1 = 48$ df. Therefore, the null hypothesis could not be rejected at the 5% level of significance and the two samples can be combined.

Spawning frequency of the day-1 females was 0.1726 with a variance of 2.3248×10^{-4}, a standard deviation of 0.0152, and a coefficient of variation of 0.0883 (Table 1). Spawning frequency of the day-2 females was 0.1481 with a variance of 1.7224×10^{-4}, a standard deviation of 0.0131, and a coefficient of variation of 0.0886 (Table 1). After joining the data from day-1 and day-2 females, the

Table 1.—Mean percentage of hydrated, day-1, day-2, and day-1/day-2 females with variance (V), standard deviation (SD), and coefficient of variation (CV).

Reproductive stage of females	Mean	V	SD	CV
Hydrated	0.2306	1.2198×10^{-3}	0.0349	0.1515
Day-1	0.1726	2.3248×10^{-4}	0.0152	0.0883
Day-2	0.1481	1.7224×10^{-4}	0.0131	0.0886
Day-1 + Day-2 (combined)	0.1604	1.0175×10^{-4}	0.0101	0.0629

combined spawning frequency was 0.1604 with a variance of 1.0175 $\times10^{-4}$, a standard deviation of 0.0101, and a coefficient of variation of 0.0629 (Table 1). The combination of day-1 and day-2 females reduced the coefficient of variation by nearly a third. This would be important for estimates of spawning biomass when using the SWFC egg production method, because the coefficient of variation of the total biomass estimate would also be reduced (Santander et al. 1984).

The spawning frequency value of 16.04% (Table 1) means that in August/September 1981 a mature Peruvian anchovy female spawned a new batch of eggs every 6.23 days, on average. The high daily incidence of 23.06% of hydrated females (Table 1) clearly shows the oversampling of females in the hydrated stage. Comparison of the coefficient of variation of the hydrated females with that of the day-1 and day-2 females demonstrated much higher variability in the occurrence of hydrated females in the samples.

DISCUSSION

The midwater trawl is reportedly a biased sampler with respect to sex ratio and hydrated females (Hunter and Goldberg 1980; Stauffer and Picquelle 1981). A higher than expected number of trawl samples had either a high or a low number of females. Furthermore, hydrated females were twice as numerous as day-1 females. Picquelle and Hewitt (1983) suggest that males and hydrated females segregate from other females at the hours of peak spawning at a depth where they are more vulnerable to the trawl.

The purse seine also seems to collect samples which are biased with respect to sex ratio and hydrated females (Alheit et al. 1984). As for the trawl, this is complicated by the fact that both biases are interrelated. In the present study, oversampling of hydrated females occurred in the early morning hours when the onset of hydration could be determined only by recording the migration of the oocyte nucleus to the pole. At this time, the oocytes have not increased perceptibly in size and the ovary is a small fraction of the total female body weight. Three different explanations are possible for the oversampling of hydrated females in purse seines:

1) Hydration decreases the ability of female anchovies to avoid nets;

2) Females segregate vertically (by depth) and those with hydrated oocytes are more accessible to the purse seine than other females;

3) Females segregate horizontally (by area) and those with hydrated oocytes occur in different areas than those without hydrated oocytes.

In any case, the suggestion by Hunter and Macewicz (1980) to use incidence of hydrated females for determination of spawning frequency seems unfeasible with purse seine collections, even if day samples of anchovies can be obtained.

The sex ratio in the 49 Peruvian collections ranges from 12.62% to 90.50% females with an average of 56.43%. Night purse seine collections contain a high percentage of hydrated females (with respect to the female fraction) and also a high percentage of males. Hydrated females are oversampled day and night, but co-occurrence of high percentages of hydrated females and males is recorded only at night. It might be hypothesized that the high male ratio in these night collections is the result of hydrated females about to spawn, which are attractive to and surrounded by a high number of males (Hunter and Goldberg 1980). If the hypothesis is correct that hydrated females are caught more often than expected because of their increased vulnerability to the net, then only the hydrated females should be oversampled and not the males as well. Thus it seems more likely that hydrated females segregate, either by depth or by area, from "normal schools" taking a high percentage of males with them, thereby forming "spawning schools" which are dominated by males.

An average sex ratio in the Peruvian survey (Alheit et al. 1984) was biased by night collections with a high percentage of hydrated females. When these collections were omitted, the average sex ratio rose to 57.9%. Assuming a sex ratio of 50% females, the purse seine clearly oversamples females (Alheit et al. 1984) whereas the trawl seems to undersample them slightly (Stauffer and Picquelle 1981; Picquelle and Hewitt 1983). Klingbeil (1978) reports similar findings when he compared the sex ratios obtained from commercial purse seiners (females:males = 1.60:1) and from research trawlers (females:males = 1.09:1). He suggests that the male-dominated schools may not form the large dense aggregations necessary for effective purse seining, as do the other schools. This might explain the difference in sex ratios estimated for the northern and Peruvian anchovy. The large purse seiner used in Peru may have shot the seine when the echosounder indicated a relatively large, dense female-dominated school. However, the California trawler took samples over a larger area and thus included more male-dominated schools which had segregated for the daily spawning period and which had formed rather loose, less dense, aggregations. Therefore, if the hypothesis is correct that spawning schools segregate by area (horizontally) from normal schools, it would seem that the trawl is a more suitable tool for determining the sex ratio of anchovies.

Interpretation of the different sampling results for hydrated females is rather difficult. They were oversampled by the Peruvian purse seiner in 1981 and by the California trawler in 1980 and 1981 (Stauffer and Picquelle 1981; Picquelle and Hewitt 1983). However, in 1982 the California trawler did not oversample the hydrated females (Picquelle and Hewitt 1983) which, according to the authors, might have been due to fishing the net at a shallower depth. This oversampling problem of hydrated females might be solved by a comparative study between a purse seiner and a larger, midwater trawl which allows fishing in depths not reached by a purse seine.

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Preservation of Northern Anchovy in Formaldehyde Solution

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ABSTRACT

Preservation and curation techniques used for northern anchovy, *Engraulis mordax*, taken in routine biomass surveys are described, and the effects of formaldehyde solution on length and weight are documented. Preservation in 10% buffered formaldehyde solution caused a 4% increase in anchovy wet weight after 24 h, but only negligible changes occurred over the next 340 d. Preservation also caused a 2% shrinkage in standard length after 24 h which increased to 3% after 340 d.

INTRODUCTION

The fish reproductive parameters used in the egg production biomass estimate (spawning fraction, female weight, batch fecundity, and sex ratio) are based on analysis of formalin-preserved specimens taken in trawl or purse seine collections. Careful attention to preservation and curation techniques is essential for an accurate measurement of these variables.

PRESERVATION

Histological analysis requires special care in preservation. Postovulatory follicles are relatively subtle histological structures, and poor preservation makes it impossible to stage them. Fish should be preserved while alive. Neither frozen specimens nor fish dead for some time can be used. A limited amount of biological material is preserved per collection container. We preserve only five adult anchovies per quart jar, roughly 80-150 g of tissue in 800 ml of formalin (see following). In all specimens, the body cavity is slit open along the side using a long incision to insure adequate preservation of the ovary. Care must be taken not to cut the ovary; this is difficult in females with hydrated eggs. If eggs are lost, they should be added to the jar.

To insure adequate preservation of the ovaries, we use a 10% buffered formaldehyde solution (formalin). Quantities of chemicals required for an 18-L container of buffered 10% formaldehyde preservative are as follows:

16.2 L distilled water
117 g sodium phosphate dibasic (granular, $Na_2H_2PO_4$)
72 g sodium phosphate monobasic (granular, $NaH_2PO_4 \cdot H_2O$)
1.8 L formaldehyde solution (37%).

Dissolve the sodium phosphate diabasic and stir in the distilled water; then mix in the sodium phosphate monobasic until dissolved, and finally add the formaldehyde solution.

Although Bouin's solution (Galigher and Kozloff 1971) might improve the quality of histological sections of ovarian tissue, its use is impractical for production work. Bouin's is more difficult to use on shipboard because represervation is required and because Bouin's preservation makes fecundity estimation difficult. Specimens preserved as described above are kept in the original preservative until curated, which may occur after as much as 2-3 mo of storage.

CURATION

A total of 15-25 females with active ovaries are removed from each collection in random order, blotted, weighed (nearest 0.1 g), and length measured (nearest 1 mm). We curate five more females per collection than needed for the spawning frequency estimate (see Picquelle 1985) in order to allow for females that will be rejected after histological analysis because of immaturity. (It is easier to curate a few additional females than to return to the original collection to process additional fish.) After a female has been weighed, the ovary is removed, blotted dry, and weighed to the nearest mg. Females may be discarded at this point, but the ovary is represerved in fresh buffered formaldehyde solution and stored individually in a vial. Ovaries that appear to be hydrated are noted, as are the presence and quantity of loose eggs in the jar. Each female is assigned a unique identification number (collection number plus fish number).

In female anchovy of 12 g or less, we consider ovaries immature when the ratio of ovary weight/female weight (less ovary) is 0.01. For females of 12 g or less, the probability of maturity is less than 5% when the gonad body weight ratio ≤0.01 (Hunter and Macewicz 1980). These ovaries are stored in vials, but not processed histologically, regardless of the size of the ovary. Histological criteria are used to distinguish between immature and inactive ovaries in females heavier than 12 g (Hunter and Macewicz 1985).

Males are processed along with the females if a separate sample is not taken for sex ratio estimation (Picquelle 1985). If males and females are preserved together, fish must be taken randomly from the jars to maintain a random order by sex. Males are weighed until the combined total of male plus female weight in a single sample equals 500 g (the combined weight needed for sex ratio calculations), and thereafter no additional males are processed in that sample.

Table 1.—Effect of formalin preservation on standard length and wet weight of Northern anchovy preserved alive.

Elapsed time in formalin (Days)	Percent change from live measurement	
	Length (mean, 2×SE*)	Weight (mean, 2×SE*)
1	−2.26 0.21	4.20 0.54
5	−2.44 0.24	4.20 0.59
10	−2.55 0.24	4.26 0.60
21	−2.81 0.24	5.40 0.74
42	−2.46 0.23	4.35 0.68
84	−2.27 0.21	3.70 0.74
341	−2.93 0.23	3.74 0.72

*Two times the standard error of the mean, where n for each sample = 55, except at elapsed time 21 days where n = 38.

EFFECTS OF PRESERVATION

The reproductive parameters used for biomass estimation are expressed in terms of live wet weight, but as the specimens are preserved in formaldehyde solution, a correction coefficient must be used.

The wet weight and length of 75 northern anchovy (ranging in weight from 2.6 to 27.0 g; mean weight 14.03 g) were determined when alive, 24 h after formaldehyde preservation, and at intervals over 340 d. Fifty-five fish were treated using the standard method described above (preserved alive with body cavity slit), 10 fish were first pithed, body cavity slit, and preserved while still alive, and 10 were pithed, slit, and allowed to die over a 20-min period before preservation.

In nearly all fish, the wet weight increased as a result of preservation. In only one of the 75 fish preserved was the wet weight after 85 d less than the initial live wet weight, and in only four fish was the weight the same. The increase in wet weight was minor. The mean increase in wet weight of the 55 fish preserved using the standard method was about 4%. In these fish, the total gain in wet weight occurred within 24 h after preservation and thereafter it remained about the same (Fig. 1). Pithed anchovy did not struggle in the preservative, and the initial weight gain after preservation was only 1%; but they gained weight steadily, attaining a 3% gain in 20 d and thereafter weight remained about the same. No difference existed between the two groups of pithed fish, indicating that death itself had no effect on subsequent weight in formalin. Struggling of live fish in formaldehyde solution appears to increase the initial rate of fluid uptake, but after 20 or more days the difference between fish preserved alive and those pithed or dead was 1% or less. This could cause a slight error in estimated live weight if some fish were preserved dead and others alive and the collection was curated immediately. For biomass estimation, we use the mean for all data on fish preserved alive (+4.3%). Most fish in our surveys are preserved alive and weighed 30-40 d later.

The length of all fish declined as a result of formalin preservation. Shrinkage after 24 h in preservative was 2% and increased to 3% after 340 d in preservative (Table 1). Shrinkage rates did not vary among the three treatment groups.

Similar effects of formalin preservation (shrinkage in length and gain in wet weight) were documented for salmon by Parker (1963), but plaice lost 9% of their wet weight when preserved in 4% formalin and sea water (Lockwood and Daly 1975), and Rosenthal and Westernhagen (1976) report no significant change in three species of siganids after formalin fixation. These and other studies (see reviews by Parker 1963, and Lockwood and Daly 1975) indicate that considerable variation exists with formalin preservation. Factors that account for this variation include species differences, fish size (life stage), state of the fish when preserved, duration of the fish in formalin, and ratio of formalin and dilutant. Among dilutants, fresh water is clearly preferable to sea water (Lockwood and Daly 1975). Clearly, the effects of formalin preservation are quite specific and calibration is required for any changes in technique, species, or life stage.

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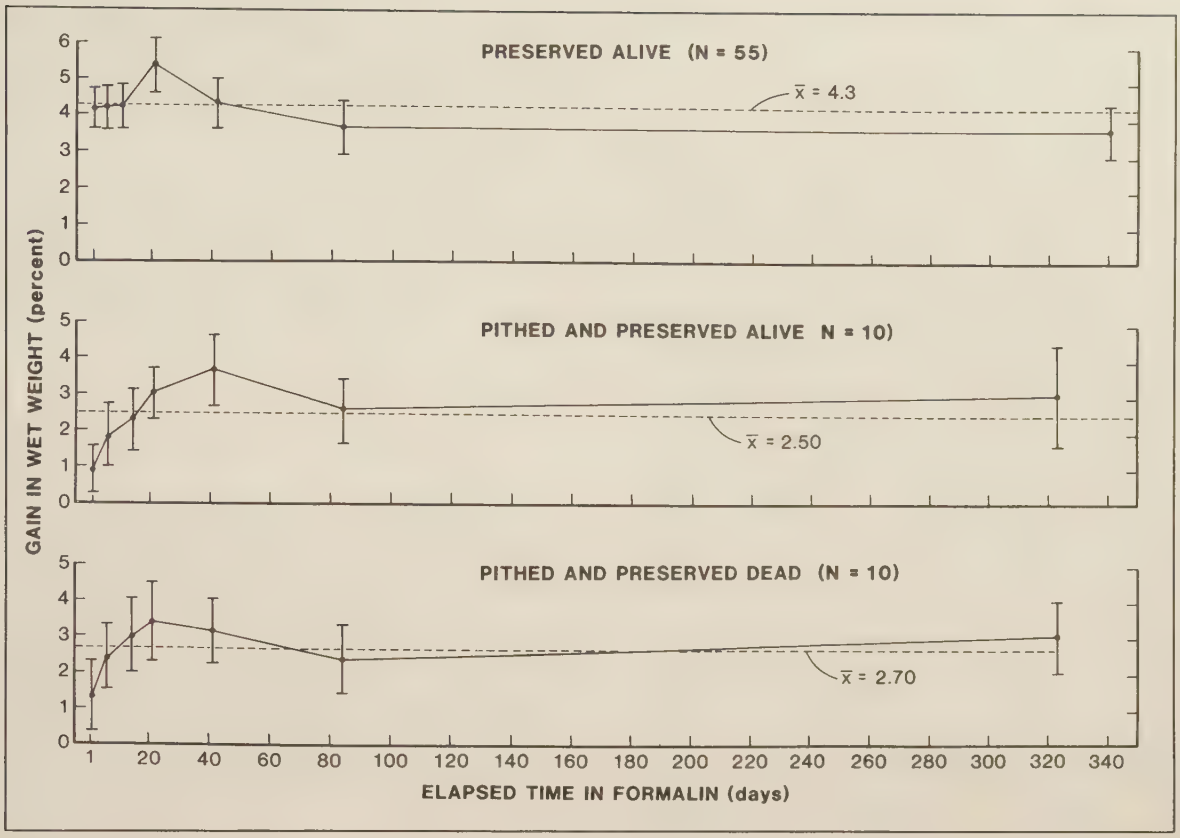
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x̄ = 4.3
PITHED AND PRESERVED ALIVE N = 10)
x̄ = 2.50
PITHED AND PRESERVED DEAD (N = 10)
x̄ = 2.70
GAIN IN WET WEIGHT (percent)
ELAPSED TIME IN FORMALIN (days)

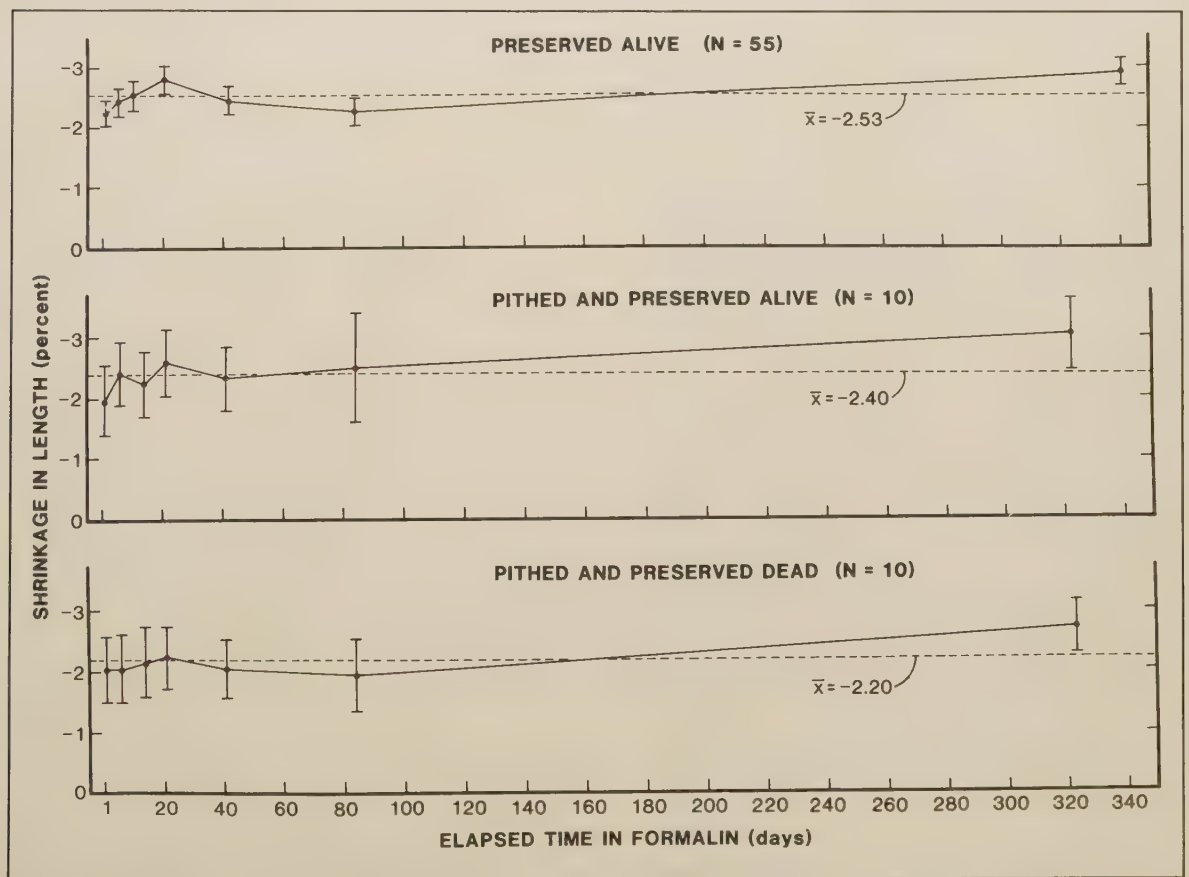
PRESERVED ALIVE (N = 55)
x̄ = −2.53
PITHED AND PRESERVED ALIVE (N = 10)
x̄ = −2.40
PITHED AND PRESERVED DEAD (N = 10)
x̄ = −2.20
SHRINKAGE IN LENGTH (percent)
ELAPSED TIME IN FORMALIN (days)

Figure 1.—Effect of 10% formaldehyde preservation on northern anchovy wet weight (upper) and standard length (lower); points are wet weight (upper) or mean loss in standard length (lower) expressed as a percentage of the original live wet weight or live length; bars are ±2×standard error of the mean.

Batch Fecundity in Multiple Spawning Fishes

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ABSTRACT

Methods for estimating batch fecundity are described, including identification and sampling the eggs that constitute one spawning batch within the ovary. Using northern anchovy, *Engaulis mordax*, as an example, methods are developed for evaluating the accuracy and precision of batch fecundity estimates. Included in this analysis are the number and location of ovarian tissue samples, optimal numbers of females, and selection of the appropriate function to express the relation between batch fecundity and female weight. We found for anchovy that the optimum number of ovarian tissue samples was 2-3 per ovary and that to keep the coefficient of variation for the mean fecundity of population under 10% required a sample of 50 or more females. Analysis of covariance indicated that the batch fecundity of northern anchovy varied significantly among years (1951-84) indicating that the relation between female weight and fecundity must be newly established each year.

INTRODUCTION

A key issue in the estimation of fecundity of oviparous fishes is whether or not the annual fecundity can be estimated from the standing stock of advanced oocytes in the ovary prior to the onset of the reproductive season. In some boreal species, frequently called total or isochronal spawners, all the eggs to be released in a season develop synchronously prior to spawning (hence the term isochronal) and spawning typically takes place over a short period (Holden and Raitt 1974). In such species, the standing stock of oocytes within a certain range of maturity classes is considered to represent the annual fecundity of the spawner. The groups of oocytes to be spawned in the season are usually identifiable because a distinct hiatus in oocyte maturity classes exists between the small, immature, unyolked oocytes that occur the year around and the synchronously maturing annual batch (Hickling and Rutenberg 1936; Yamamoto 1956). Although some of these fishes may spawn repeatedly during the season, for example, whiting and haddock, the standing stock of yolked eggs is considered representative of the annual fecundity (Hislop 1975; Hislop et al. 1978; Hislop, pers. commun.[1]). An exception to this occurs when unfavorable conditions result in resorption of some of the advanced eggs in the ovary at the end of the season. The extent of this potential bias (overestimation of annual fecundity) is unknown.

In many temperate and tropical fishes (frequently called multiple, partial, serial, or heterochronal spawners), annual fecundity is seasonally indeterminate and batch fecundity is the only useful measurement. In such fishes the standing stock of yolked eggs, regardless of maturity state, give no indication of annual fecundity because these fishes continuously mature new spawning batches throughout a typically protracted spawning season. In the active ovaries of fishes with indeterminate annual fecundity, the oocytes usually occur in nearly all maturity stages; they range in size continuously from small unyolked oocytes <0.1 mm diam. to yolked oocytes 0.4-0.7 mm diam., and no large hiatus exists between maturity classes of oocytes except for one between hydrated oocytes and advanced yolked oocytes which is of a temporary nature. Such fishes usually spawn many times during a season. The northern anchovy spawns at 7-10 d intervals for 2 or 3 mo and averages 20 spawnings per yr (Hunter and Leong 1981), and the scianid, *Seriphus politus*, has a similar reproductive output (DeMartini and Fountain 1981). Thus, for these fishes, identification of a predetermined annual spawning batch is a hopeless exercise, and the only useful fecundity measurement is the number of eggs produced in a single spawning batch (batch fecundity); annual fecundity is a function of both the batch fecundity and the number of spawnings per year. Spawnings are so numerous in these fishes that small unyolked oocytes <0.1 mm diam. would have to mature in a season to account for the number of spawnings (Hunter and Leong 1981).

The standing stock of oocytes is occasionally used to estimate annual fecundity in such common fishes as *Scomber*, *Trachurus*, and *Merluccius*, which by the standard criteria have indeterminate fecundity. That annual fecundity is predetermined in such fishes is an assumption with little or no supporting evidence. The criteria and approaches for distinguishing between determinate and indeterminate fecundity are discussed in greater detail (Hunter and Macewicz 1985).

The objective of this paper is to describe the methodologies for estimating batch fecundity in fishes with indeterminate seasonal fecundity. We do not consider the well documented methodology

[1]John R. G. Hislop, DAFS Marine Lab., P.O. Box 101, Victoria Road, Aberdeen AB9 8DB, Scotland, pers. commun. Oct. 25, 1983.

for fecundity estimation of fishes with seasonally determinate fecundity (see, for example, Holden and Raitt 1974). In anchovy and other fishes with indeterminate annual fecundity, the oocytes in active ovaries are typically distributed in 1-2 modes (Fig. 1), each mode representing a single spawning batch. Maturation of oocytes and vitellogenesis are a continuous cycle: when one spawning batch is spawned, another spawning batch is ready for the last stages of maturation and spawning (Fig. 2). Vitellogenesis proceeds rapidly after a spawning, with the ovary doubling in dry weight during the interval between spawnings (Hunter and Leong 1981). The final stage of maturation, hydration, is characterized by a rapid secretion of fluid of low specific gravity into the advanced eggs by the granulosa cells of the follicle (Fulton 1898). This fluid causes more or less complete fusion or solution of the yolk granules producing the translucent appearance of hydrated eggs. The volume of the egg or wet weight increases three- or four-fold (Fulton 1898), but the increase in dry weight is negligible (LeClus 1979a). In northern anchovy, hydration begins about 12 h before spawning when the eggs are between 0.6 and 0.8 mm (major egg axis) and causes a four-fold increase in wet weight of the ovary as the egg increases to 1.3 mm (major axis) (Hunter and Macewicz 1980; Hunter and Leong 1981) (Fig. 2). Ovulation and spawning soon follow completion of hydration in most clupeoids (anchovy, pilchard, sardines, and others) but in herring, a total spawner, ovulated eggs may be retained in the ovary for an extended period.

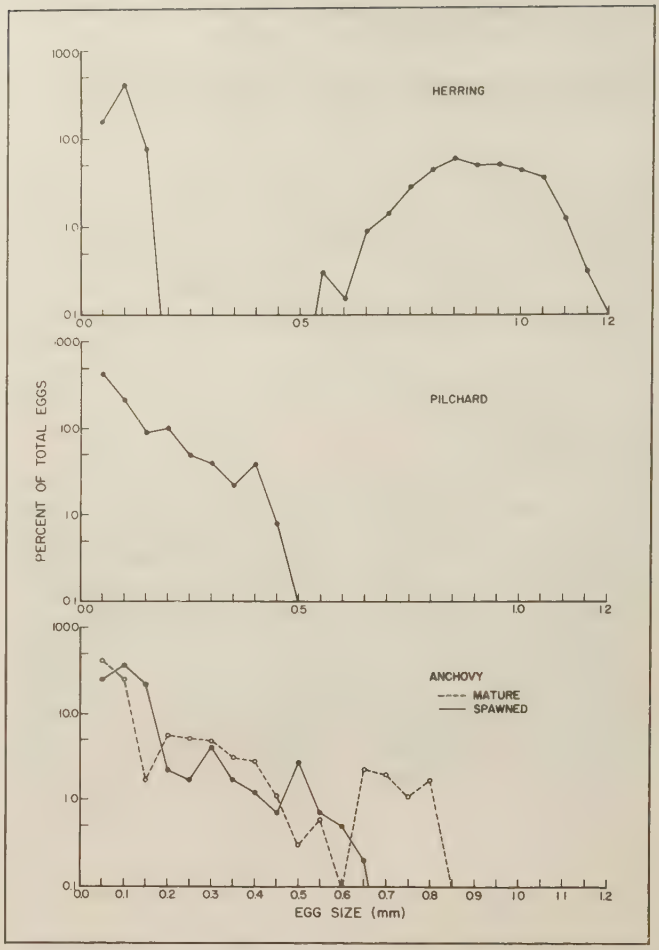

Figure 1.—Frequency distribution of oocyte diameter in the ovaries of herring, pilchard (Hickling and Rutenberg 1936), and northern anchovy (Hunter and Leong 1981). Herring spawns a single batch each year; other species are multiple-batch spawners. In the anchovy, the solid line shows a recently spawned female, the broken line a female about to spawn (just before hydration of the oocytes). (From Blaxter and Hunter 1982.)

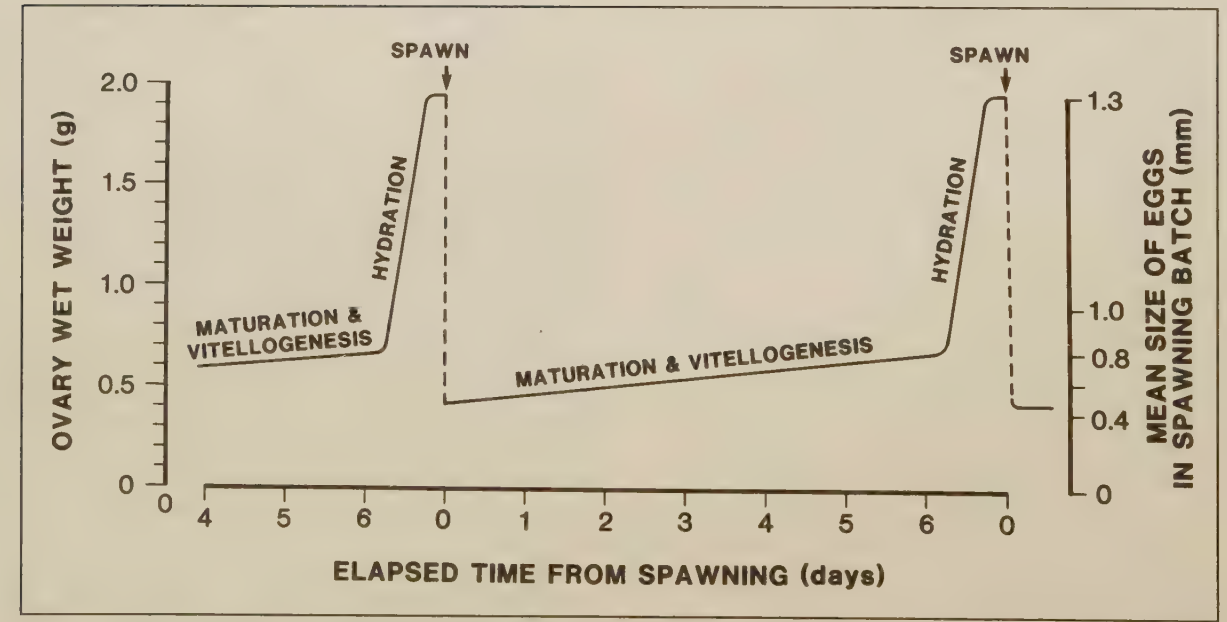

Figure 2.—Maturation cycle of a 12.5 g northern anchovy female during peak spawning months where the average interval between spawnings is 7 d. The change in mean wet weight of the ovary is indicated on the left axis and the mean diameter of the oocytes in the most advanced spawning batch on the right axis. Data from Hunter and Goldberg (1980) and Hunter and Macewicz (1980).

LABORATORY PROCEDURES

Identifying and Counting Oocytes in a Batch

A number of criteria have been used to identify the oocytes to be included in a spawning batch. These include 1) counts of all yolked oocytes; 2) estimation of the number of oocytes in the most advanced spawning batch by measuring the size distribution of oocytes in the ovary and identifying the most advanced (largest) modal group of oocytes; and 3) estimation of the number of oocytes in a spawning batch by counting the number of hydrated oocytes.

The first method can be rejected because the standing stock of yolked oocytes gives indication neither of total fecundity nor of batch size in fishes with indeterminate fecundity and is appropriate only for fishes with determinate seasonal fecundity. The second method (oocyte size frequency) usually gives results similar to those based on counts of hydrated oocytes if females with highly advanced oocytes are used (Hunter and Goldberg 1980; Laroche and Richardson 1980). We believe the third method, counting the hydrated oocytes, is preferable because it requires less time and avoids the problem of partitioning oocytes between the most advanced mode and the adjacent group of smaller oocytes. Before describing methods 2 and 3, it is important to consider methods of sampling the ovary.

Methods of Sampling an Ovary

It is impractical to count and measure all advanced oocytes, or to count all hydrated oocytes in an ovary, owing to the great fecundity of most marine fishes. Thus, regardless of the method used for identifying the spawning batch, ovarian subsamples are required, and these are related to either the ovarian weight (gravimetric method) or the total volume of an aqueous suspension of all oocytes in the ovary (volumetric method). The gravimetric method is based on counting oocytes in weighed samples of ovarian tissue and relating the tissue samples to the total ovary weight. In the volumetric method, the ovary is preserved in Gilson's fluid which frees the oocytes from the ovarian tissue by breaking down the connective tissue (see Baganel 1967 for the recipe for Gilson's fluid). The released oocytes are cleaned, put in a volumetric cylinder filled to a known volume with water, shaken to provide thorough mixing, subsamples of known volume are withdrawn using a Stempel pipette, and the oocytes staged and counted (Holden and Raitt 1974). Automatic oocyte counters may also be employed.

The volumetric method may be used for batch fecundity estimation if the eggs constituting the batch are identified using the egg size-frequency method; however, the volumetric technique is inappropriate if the hydrated oocyte method is used because Gilson's fluid destroys hydrated eggs. Substantial shrinkage of oocytes occurs when ovaries are preserved in Gilson's fluid; an average shrinkage of 24% (compared to formalin-preserved ovaries) occurs when skipjack and yellowfin tuna ovaries are preserved in Gilson's fluid, but no differential shrinkage occurs among oocyte size classes (Joseph 1963). Thus, to make oocyte size classes comparable to live, or formalin-preserved, or histological sections, the extent of shrinkage must be measured and the data corrected. Treatment with Gilson's fluid also destroys the ovary, making histological analysis impossible.

We use the gravimetric method of MacGregor (1957), which is somewhat similar to the gravimetric method "B" of LeClus (1977) who evaluated two gravimetric and one volumetric techniques. Although LeClus obtained very low coefficients of variation for all techniques in her 1977 methods paper, the relative fecundity estimated for the South African anchovy in a subsequent paper (LeClus 1979b) was as variable as those employing less complicated procedures (MacGregor 1957, 1968; Hunter and Goldberg 1980). Her use of vacuum dry weight of the ovary, instead of formalin wet weight, seems an unnecessary refinement. Natural variability in batch fecundity appears to be much greater than the variation caused by differences in the technique of sampling the ovary.

The step-by-step procedure for the hydrated oocyte method is outlined below. It will be elementary to many biologists, but it is intended as a guide for inexperienced staff. Except for some details outlined in a subsequent section, the same procedure can be used to estimate batch fecundity using the oocyte size-frequency method.

The Hydrated Oocyte Method

1. The basic method is as follows: Numbers of hydrated oocytes in weighed tissue samples of formalin-preserved ovary are counted and the counts are then projected to estimate numbers of hydrated oocytes in the entire ovary which is assumed to be equivalent to batch fecundity.

2. Prior to batch fecundity estimation, females are accurately weighed, and the ovary removed, weighed, and stored in an individual vial of buffered 10% formalin. Ovaries which appear hydrated are noted along with an estimate of the numbers of free eggs in a collection jar (Hunter 1985). Only hydrated ovaries which have not lost oocytes are used for fecundity estimation; ovaries that have lost oocytes in the jar are rejected after histological examination because they contain postovulatory follicles.

3. Needed supplies and equipment include a balance sensitive to 0.1 mg (which should be checked with standard weights), a dissection microscope with a 10× objective, hand counter, forceps, scalpel, bottle of glycerin (33% glycerol solution by volume) with eyedropper, glass slides (25×75 mm), cover slips (22×50 mm), paper towels for blotting, and weighing paper.

4. Remove ovary from the formalin fixative and blot dry with paper towel. Break the ovarian membrane and remove three tissue samples of the ovary. The ovary is soft and the sample can be removed easily with the tip of the forceps or scalpel. Remove samples from positions about one-third of the distance from each end of the ovary to insure that no two samples come from the same portion of the ovary (only one ovary, left or right, need be used). Try to obtain a tissue weight of 30-50 mg, as this will contain an adequate number of hydrated oocytes (100-200). Place sample on a preweighed piece of weighing paper and record weight to nearest 0.1 mg. Pieces of ovary can be added or removed to vary sample weight.

5. Place the sample on a slide and cover with 3-4 drops of glycerin. After 10-15 min, loosen the oocytes by gently tapping the piece of ovary with the blunt tip of the forceps. After the oocytes are loosened, add 3 or 4 more drops of glycerin, spread the sample over the slide, and cover with a cover slip so that it floats on the fluid. (We found that this concentration of glycerin had no effect on the diameters of oocytes taken from formalin-preserved ovaries, even after 24 h.)

6. Place the slide under the microscope, and with a hand counter tally the number of hydrated oocytes in the sample. Hydrated oocytes can be distinguished easily from other oocytes by their large size

(usually ≥0.8 mm in the major axis in northern anchovy), wrinkled appearance when formalin preserved (yolked but nonhydrated oocytes usually retain their smooth surface contour), and by their translucence (nonhydrated eggs are relatively opaque, Fig. 3). Some damage to the hydrated oocytes may occur during slide preparation. In some cases, the chorion may be ruptured and the yolk extruded. Do not count empty chorions, and count only those fragments judged to be major portions of the oocytes.

7. Batch fecundity (Y) for each female is calculated from the product of the number of hydrated oocytes (eggs) per unit weight in the tissue sample and the ovary weight (left and right sides combined) (Z).

8. The egg production method requires that batch fecundity be expressed as a function of female weight and not length, i.e., $EY = f(w)$ where female weight (w) is the formalin wet weight of the female without the ovary (formalin wet weight can be converted to live weight using coefficients given in Hunter 1985). We use ovary-free wet weight of females since females with hydrated ovaries temporarily have a higher weight than the average female because of the increased weight of the hydrated ovary. The ratio of female body weight without ovary to female weight with ovary (excluding females with hydrated or immature ovaries) can be used to convert ovary-free wet weight to total body weight. This ratio in northern anchovy was 0.95 for female anchovy taken 1978-79 (Hunter and Macewicz 1980).

Oocyte Size-Frequency Method

If the number of females with hydrated oocytes is insufficient for a batch fecundity estimate, the more time-consuming oocyte size-frequency distribution method can be employed (MacGregor 1957). This method takes 1-3 h per fish (3 tissue samples per fish) as compared with 1-1.5 h for the hydrated oocyte method. In this method, a size-frequency distribution of oocytes is constructed and the most advanced modal group of oocyte size classes (the mode composed of the largest oocytes) is determined by inspection. The total number of oocytes within the oocyte size classes that constitute the advanced modal group is considered to be the spawning batch. This method usually gives results similar to those based on counts of hydrated oocytes if females with highly advanced oocytes are used (Hunter and Goldberg 1980; Laroche and Richardson 1980). The mean size of the group of oocytes that constitutes the most advanced spawning batch should be ≥0.5 mm, as estimates of batch fecundity are somewhat inflated if a less mature ovary is used (Hunter and Goldberg 1980).

In northern anchovy, a tissue sample weight of ≤10 mg will insure that about 100 oocytes are included in the most advanced modal group of oocytes. All oocytes ≥0.3 mm in a tissue sample are counted using a set of hand counters and measured to the nearest 0.05 mm. We use an optical comparator at 50× magnification and measure the oocytes with a rule on the viewing screen of the comparator. A starting oocyte size ≤0.3 mm is recommended to insure that a

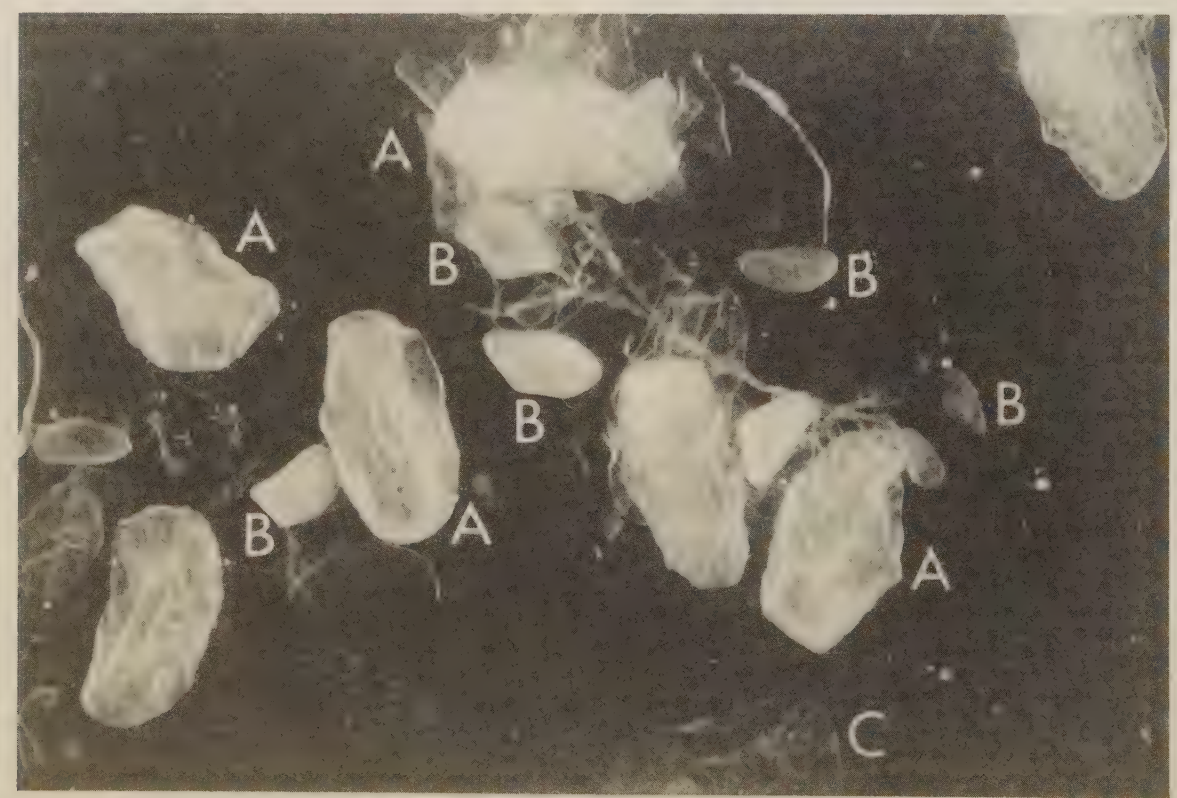

Figure 3.—Hydrated oocytes in a tissue sample taken from a northern anchovy ovary preserved in formaldehyde solution. A. Hydrated oocytes; B. Unyolked and yolked oocytes (before hydration); C. Empty chorion of a hydrated oocyte (the chorion, or a major fragment of it, would be included in the count of hydrated oocytes for the batch fecundity estimate). These hydrated oocytes were 1.2 mm long in the major axis.

sufficient number of 0.05-mm oocyte size classes exist below the most advanced modal group. Inclusion of these small oocyte size classes insures an accurate separation of the tail of the advanced mode of oocytes from the smaller oocytes adjacent to it. To separate the tail of the advanced modal group from the adjacent group of smaller oocytes, we use probability paper analysis (Harding 1949; Cassie 1954). In other respects, the oocyte size-frequency method is the same as the hydrated oocyte method, and the previous section can be used as a guide.

ACCURACY AND PRECISION

In this section we develop methods to evaluate the accuracy and precision of estimating batch fecundity using the hydrated egg methodology, and apply these procedures to northern anchovy fecundity data. Accurate estimation of batch fecundity depends upon selection of an unbiased location for the samples of ovarian tissue and the selection of an appropriate regression model to express the relation between female weight and batch fecundity. The precision of the estimate depends upon the number of ovarian tissue samples taken per female and the total number of females. The weight of the individual tissue sample also affects precision, but we have not considered this element. We have instead kept the tissue samples within a weight range that yields about 100-200 hydrated oocytes per sample.

In the first subsection we use analysis of variance to detect the possible effects of location of tissue samples within the ovary. In this analysis we use a sample of 12 northern anchovy ovaries in which 6 tissue samples were taken per ovary at specified locations. In the next subsection we determine how the number of tissue samples affect the precision of the fecundity estimate; consider various fecundity-weight models; and determine the optimum numbers of fish and tissue samples for a given cost. For this analysis we add an additional 12 fish to the sample used in the first section, all of which had 6 tissue samples per fish. In the third subsection we use seven data sets on anchovy batch fecundity taken 1951-60 and 1978-84 (n ranges, 19-127) to validate selection of the fecundity-female weight model and to assess the precision of regression estimates of batch fecundity. In the final subsections we consider how the number of fish in the sample affects precision of the fecundity estimate and how the batch fecundity varies among years.

Location of Tissue Samples Within the Ovary

To determine if location of the tissue samples affects estimates of batch fecundity in anchovy, we took 6 ovarian tissue samples from each of the 12 ovaries; three samples were taken from the left ovary and three from the right. In each set of three, one sample was taken in the center and the other two were about one-third of the distance from each end of the ovary. The number of eggs per unit weight of the ovary (x) was calculated for each tissue sample. We tested effects of right or left ovarian side, and position of tissue samples within a side, using the two-way analysis of variance. The natural logarithm of x was used in the analysis because there is a positive correlation between the same sample mean and its standard deviation. The assumption of homogeneity within sample variance is violated when the means differ.

No difference existed either in the location of the tissue sample within right or left sides of the anchovy ovary or between right and left sides (Table 1). Thus in northern anchovy, tissue samples can be taken from any location or from either the right or left sides. It should be noted, however, that all of our samples were of females taken at a time of day (1900-0100 h) when hydration was nearly complete. If females are taken earlier in the day, position effects may be likely because hydration does not proceed at a uniform rate throughout the ovary; rather it begins at the periphery and spreads to the central section of the ovary, producing larger hydrated oocytes (more mature) at the periphery than in the center. Thus the number of hydrated oocytes per gram of ovary may be higher in the central section and lower on the periphery at early stages of hydration. Variation in extent of hydration does not appear to be a major source of error, but it should be evaluated prior to making a fecundity estimate in a new species or when samples are taken at a new time of day.

Table 1.—Effect of location of ovarian tissue samples from northern anchovy on the number of hydrated eggs per unit sample weight (g). Effects evaluated by taking tissue samples (n = sample size) from three positions (two ends, I and III, and middle, II) from both the right and left ovary. Analysis of variance indicates insignificance of either side or position within a side (SS = sum of squares; MS = mean square).

Positions of sample in ovary	Mean no. of eggs/g of ovary tissue								
	Right ovary			Left ovary			Both ovaries		
	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n
I	5,237	740	12	5,143	1,158	12	5,190	950	24
II	4,919	790	12	5,243	831	12	5,081	810	24
III	5,296	1,005	12	5,540	2,019	12	5,418	1,564	24
All	5,151	845	36	5,309	1,396	36			

Two-way analysis of variance of eggs/g of ovary tissue.

SS due to	df	SS	MS	F
Right vs. left ovary	1	0.0053	0.0053	0.14
Position within ovary	2	0.0268	0.0134	0.34
Interaction	2	0.0269	0.0134	0.34
Error	66	2.5854	0.0392	—
Total	71	2.6444	—	—

Optimum Number of Tissue Samples and Numbers of Fish for Fecundity Estimation

In this section we develop equations to estimate the optimum number of fish and tissue samples to be used for fecundity estimation using data on the northern anchovy. The precision of the sample variance (or mean square error) around the regression of batch fecundity on female weight (σ_A^2) (a measure of the goodness of fit of the fecundity-weight model) was used to determine the optimum number of tissue and fish samples. This procedure required a definition of a general fecundity model and development of functions to express the error terms.

A. The general fecundity model—The true batch fecundity (Y) where all eggs are counted, and the fish weight relationship (w) is defined as:

$$Y_w = f(w) + A \qquad (1)$$

where the error term (A) has a mean = 0, and variance = σ_A^2. Y and Y_w are interchangeable in later sections. Since all the hydrated eggs in a batch (Y) are not counted, $f(w)$ are fitted to the estimated batch fecundities ($\hat{Y}$) calculated from m ovarian tissue samples per fish.

Let's denote for the ith fish, $i=1,\ldots,n$:

w_i = gonad-free fish weight = fish weight minus gonad weight
Y_i = total number of hydrated eggs in the ovary
y_{ij} = hydrated egg count in the jth tissue sample $j=1,\ldots,m$
z_{ij} = weight of jth tissue sample
Z_i = formalin wet weight of gonad
m = number of tissue samples from an ovary
M_i = maximum number of tissue samples in an ovary
$\hat{Y}_{ij}$ = estimated total number hydrated eggs in the ovary from the jth tissue sample = $(y_{ij}/z_{ij})Z_i$
$\hat{Y}_i$ = estimated total number of hydrated eggs in the ovary $= \sum_{j=1}^{m} \hat{Y}_{ij}/m$
$\bar{Y}$ = sample mean number of hydrated eggs = $\sum_i \hat{Y}_i/n$
$\tilde{Y}_i$ = estimate of batch fecundity from the regression model.

Suppose $\hat{Y}_{ij}$ is an unbiased estimate of Y_i, then

$$\hat{Y}_{ij} = Y_i + \hat{Y}_{ij} - Y_i = f(w_i) + A_i + e_{ij} \tag{2}$$

where $e_{ij} = \hat{Y}_{ij} - Y_i$ is the within-ovary error term and is assumed normally distributed with mean = 0 and variance = σ_e^2. Using Equation (2) and the fact that

$$\hat{Y}_i = \sum_{j=1}^{m} \hat{Y}_{ij}/m, \text{ we have}$$

$$\begin{aligned} \hat{Y}_i &= f(w_i) + A_i + e_{i\cdot} \\ &= f(w_i) + \xi_i \end{aligned} \tag{3}$$

where $\xi_i = A_i + e_{i\cdot}$ and $\sigma_{\xi_i}^2 = \sigma_A^2 + \frac{M_i - m}{M_i}\frac{\sigma_e^2}{m}$.

Thus the variance around the regression line based upon data set $(\hat{Y}_i, w_i)$ is composed of two variance components: One is σ_A^2 and the other is σ_e^2, the within-ovary variance. The unbiased estimates of $\sigma_{\xi_i}^2$ and σ_e^2 are

$$s_{\xi_i}^2 = \frac{\sum_{i=1}^{n} [\hat{Y}_i - f(w_i)]^2}{n - q} \tag{4}$$

and

$$s_e^2 = \frac{\sum_i \sum_j (\hat{Y}_{ij} - \hat{Y}_i)^2}{n\,(m-1)}. \tag{5}$$

The parameter q is the number of regression coefficients in the model and n is the number of fish sampled. Most if not all fecundity regressions use only two coefficients (Bagenal 1967) and consequently we use $q = 2$ in this article, and subsequent computations should be redone if $q>2$. For simplicity, we assume that $M_i = M_j = M$ for $j \neq i$, and $\sigma_{\xi_i}^2 = \sigma_{\xi_j}^2 = \sigma_\xi^2$.

Thus

$$\sigma_A^2 = \sigma_\xi^2 - \frac{M-m}{M}\frac{\sigma_e^2}{m}$$

and

$$s_A^2 = s_\xi^2 - \frac{M-m}{M}\frac{s_e^2}{m}. \tag{6}$$

The variance of the sample variance (s_A^2) is used in the next section for computing the optimum number of ovarian tissue samples for fecundity estimation.

B. Optimum number of tissue samples—Since the goodness of fit of any model is measured by s_A^2, we chose to minimize the variance of s_A^2 with respect to the number of tissue samples (m) for a fixed total cost function (Scheffé 1959).

$$c = c_1 n + c_2 mn \tag{7}$$

where c is the total funds available,
c_1 is the cost of processing a fish, and
c_2 is the cost of processing a tissue sample.

Under the assumption of normality of the error terms, one obtains the variance of s_A^2 as:

$$var(s_A^2) = 2\sigma_A^4 \left[\frac{\left\{1 + \left(\frac{1}{m} - \frac{1}{M}\right)\theta\right\}^2}{(n-q)} + \left(\frac{1}{m} - \frac{1}{M}\right)^2 \frac{\theta^2}{n(m-1)} \right] \tag{8}$$

where $\theta = \frac{\sigma_e^2}{\sigma_A^2}$ and $\hat{\theta} = \frac{s_e^2}{s_A^2}$.

The optimum sample sizes for selected parameter values are listed in Table 2. The optimum tissue sample size (m) depends on the values of each of the five parameters, i.e., q, $\theta = \sigma_e^2/\sigma_A^2$, c, c_1, and c_2.

Table 2.—Optimum number of ovarian tissue samples required for batch fecundity estimation of northern anchovy with two regression coefficients (q=2) and maximum tissue samples >30 within an ovary (M>30) for various degrees of data variability ($\theta=\sigma_e^2/\sigma_A^2$) and cost constraints ($c/c_2$ and c_1/c_2).

Ratio of within-ovary variance to variance about the line ($\theta=\sigma_e^2/\sigma_A^2$)	Total funds available for estimated cost per tissue sample (c/c_2)	Relative index of processing costs (costs of fish/cost of sample (c_1/c_2)*		
		0*	4	8
θ = 0.5	50	2	2	2
	200	2	3	3
	10,000	2	3	3
θ = 1	50	2	3	3
	200	2	4	4
	10,000	2	4	5
θ = 2	50	3	4	4
	200	3	5	6
	10,000	3	6	7
θ = 3	50	3	5	4
	200	4	7	8
	10,000	4	7	9
θ = 4	50	4	6	4
	200	5	8	10
	10,000	5	9	11
θ = 5	50	5	6	4
	200	6	9	11
	10,000	7	11	13

*0 = case where cost of capturing fish is negligible relative to cost of fecundity estimate.

The finite population correction factor $(1-m/M)$ can be ignored for $M \geq 30$. This means that it is unlikely that the finite population correction will ever be used because it is very unlikely that a tissue sample as large as 1/30 of the ovary weight would ever be used in a fecundity estimation. Hence, an infinitely large M ($M \geq 30$) can be used for all calculations.

To evaluate the adequacy of Equation (3), we compute a ratio of σ_ξ^2 and σ_A^2, i.e., $K = \sigma_\xi^2/\sigma_A^2$; $K \geq 1$. A large K implies the variation in Equation (3) is too high and a larger sample size is desirable. When K is close to 1, the precision of Equation (3) is nearly as good as that of Equation (1) which uses the true fecundity where all eggs in a batch are counted. K is computed from the following equation:

$$K = \frac{\sigma_\xi^2}{\sigma_A^2} = \left(\frac{1}{m} - \frac{1}{M}\right)\theta + 1 \qquad (9)$$

and

$$\hat{K} = \frac{s_\xi^2}{s_A^2} = \left(\frac{1}{m} - \frac{1}{M}\right)\hat{\theta} + 1 \cdot$$

Table 3 gives K values for number of tissue samples ranging from 1 to 6 for $\theta = 0.5$-0.6. K can be computed easily for other values of θ and number of possible tissue samples within an ovary. The precision of $\hat{K}$ depends on the variance of s_ξ^2 and s_A^2 and the cost function for processing the fish and making the fecundity estimate [Equation (7)]. Costs dictate the number of fish used and the number of tissue samples taken per fish.

To evaluate K for different cost functions (hence sample sizes), it is necessary to select a proper function to express the relationship between fecundity and female weight, $f(w)$. We used four models and the data set of 24 fish (Table 3). The four models were:

$$\hat{Y} = \begin{cases} f_1(w) = aw^b + e \\ f_2(w) = ae^{bw} + e \\ f_3(w) = a + b\ln(w) + e \\ f_4(w) = a + bw + e \cdot \end{cases}$$

The linear model had the greatest precision because it had the lowest s_ξ^2 and s_A^2 (Table 3). The linear model also seems a preferable method of expressing the fecundity-weight relationship in northern anchovy when much larger data sets are considered (see next section).

The parameter $\hat{\theta} = s_e^2/s_A^2$ (a measure of the relative variability within tissue samples) ranged from 0.5 to 0.6, depending on the regression model selected for the 24-fish sample. In our case, the cost (c_1) of catching and curating a single fish is negligible relative to the cost (c_2) of counting the hydrated eggs in a tissue sample because many fish must be taken for the spawning frequency estimation. Thus for northern anchovy, $c_1/c_2 \sim 0$. Using Table 2, the optimum number of tissue samples (m) is 2. For m=2, and $\hat{\theta}$=0.5, $\hat{K}$=1.3 [Equation (9)]. This means that the variance around Equation (3) is about 1.3 times that of the model based on counts of all hydrated eggs in the ovary [Equation (1)]. To reduce the $\hat{K}$ value, more tissue samples are needed which increases the total cost (c). For northern anchovy, there is no reason to increase the number of tissue samples beyond three, because the reduction in $\hat{K}$ becomes negligible at larger sample sizes (Table 3).

Validation of the regression model

In the previous section we concluded that the linear model for expressing the relation of female batch fecundity to female weight was preferable, but we used a small data set (n = 24) in which 3-6 tissue samples were taken per fish. In this section we test and evaluate this conclusion by fitting a linear model ($\hat{Y}_w = \bar{Y} + b(w-\bar{w})$) and two nonlinear models ($\hat{Y} = ae^{bw}$ and $\hat{Y} = aw^b$) to all existing data sets on the fecundity of northern anchovy (1950-84) (Tables 4 and 5). In these sets the number of tissue samples per fish varied from 1 to 3. Mean square error (MSE) = $\Sigma(\hat{Y}-\bar{Y})^2/(n-2)$ was computed for all three models for each data set. Although no apparent difference existed among MSE values for the three models, and no pattern existed in the residuals (Fig. 4), the simple linear model is preferable because: 1) it explains as much variation as the cur-

Table 3.—Effect of ovarian tissue samples taken per ovary (m) on the ratio K for various models that express the relation between female weight, w (without ovary) and batch fecundity, $\hat{Y}_w$.

Regression model $f(w)$	s_ξ^2 ($\times 10^3$)	Maximum no. of tissue samples (M)	*s_A^2 ($\times 10^3$)	†s_e^2/s_A^2	‡K for 1-6 tissue samples/ovary					
					1	2	3	4	5	6
(1) aw^b	6,302	∞	5,729	0.60	1.60	1.30	1.20	1.15	1.12	1.10
a = 325.07		80	5,772	0.60	1.59	1.29	1.19	1.14	1.11	1.09
b = 1.2423		30	5,844	0.59	1.57	1.28	1.18	1.13	1.10	1.08
(2) ae^{bw}	7,112	∞	6,539	0.53	1.53	1.27	1.18	1.13	1.11	1.09
a = 3,281.28		80	6,582	0.52	1.51	1.25	1.17	1.12	1.10	1.08
b = 0.0612		30	6,654	0.50	1.50	1.24	1.16	1.11	1.09	1.07
(3) $a+b\ln(w)$	6,274	∞	5,702	0.60	1.60	1.30	1.20	1.15	1.12	1.10
a = −31,831.73		80	5,744	0.60	1.59	1.29	1.19	1.14	1.11	1.09
b = 15,371.04		30	5,816	0.59	1.57	1.28	1.18	1.13	1.10	1.08
(4) $a+bw$	6,220	∞	5,648	0.61	1.61	1.31	1.20	1.15	1.12	1.10
a = 3,190.38		80	5,690	0.60	1.59	1.29	1.19	1.14	1.11	1.09
b = 838.11		30	5,762	0.60	1.58	1.28	1.18	1.13	1.10	1.08

*From equation (6).

†Within-ovary variance (s_e^2) = 3,436×10³ from Equation (5); ratios computed using 7 digits.

‡K is a measure of the variance around regression line relative to the "free" variance around the curve (Equation (1)). When K = 1, the variance from subsampling ovarian tissue is 0 since all eggs are counted.

Table 4.—Sample statistics for batch fecundity estimation of the central subpopulation of northern anchovy for various years. (See Addendum for 1985 data.)

Statistic	1951-60*	1978	1979	1980	1981	1982	1983	1984
n†	19	23	44	33	127	109	83	87
Linear regression coefficient (b)	532	279	693	563	752	617	588	532
Linear regression intercept (a)	1,122	2,023	−4,410	−1,891	−1,979	−180	−1,002	−554
$s_{y \cdot x}$‡	2,752	3,103	2,935	1,276	2,522	2,583	1,282	1,281
Sample mean batch fecundity ($\bar{Y}$)†	10,270	7,546	8,506	7,745	9,083	10,031	5,828	5,862
Sample mean ovary-free fish weight ($\bar{w}$)†	17.18	19.76	18.64	17.10	14.70	16.54	11.63	12.06
Sample standard deviation of fish weight (s_w)	6.57	6.62	5.32	4.77	5.81	4.41	4.75	5.54
$\Sigma(w-\bar{w})^2$	777	964	1,208	728	4,253	2,100	1,850	2,639
Mean fish weight from survey ($\bar{\bar{w}}$)	—	—	—	17.50	16.20¶ 13.40	18.60	12.90¶ 11.20 9.63	12.02
Standard error of fish weight ($s_{\bar{\bar{w}}}$)	—	—	—	0.96	0.47¶ 0.52	0.37	1.56¶ 0.79 0.37	0.46
Adjusted mean batch fecundity for ovary-free fish weight = 14.95 g§	8,900	4,597	6,241	6,423	9,237	9,055	7,870	7,640
Standard error of batch fecundity for 14.95 g fish §	536	494	358	408	207	225	264	256

*From MacGregor (1968).
†Sample mean batch fecundity ($\bar{y}$) and mean fish weight ($\bar{w}$) are computed from data set with n fish.
‡Standard deviation about the line.
§Computed from analysis of covariance.
¶Computed from individual cruises (1981 values for two cruises and 1983 for three cruises).

vilinear model; 2) its regression coefficients have a simple biological meaning (b = batch fecundity/gram female weight, $\bar{Y}$ = mean batch fecundity); and 3) for the egg production estimate, the fecundities of the largest and smallest fish are not as critical as for the fish in the middle range, which is well explained by the simple linear model.

Analysis of these eight data sets also indicated that the standard deviation of the batch fecundity is a linear function of the female weight (R^2 ranges from 0.2 to 0.6). The minimum variance and unbiased estimates of the regression coefficients can be obtained through a weighted least squares regression with the inverse of the variance as the weight (Draper and Smith 1981). In most cases the standard errors of regression coefficients from the weighted least squares were smaller than those from the regular least squares.

Table 5.—Mean square error (MSE)* of linear (unweighted and weighted) and nonlinear (exponential and power function) models for relationship between batch fecundity and fish weight of northern anchovy, based on data sets by years.

	Regression models				
	Linear		Nonlinear		
Year	Unweighted ($\times 10^{-6}$)	Weighted	Exponential ($\times 10^{-6}$)	Power function	Sample size (n)
1951-60	7.6	9.0	7.3	7.5	19
1978	9.6	8.3	10.2	8.1	23
1979	6.5	7.4	7.8	8.3	44
1980	1.6	1.5	1.5	1.5	33
1981	6.4	5.6	7.1	6.4	127
1982	6.8	4.9	6.7	6.6	109
1983	1.7	1.9	1.9	1.6	83
1984	2.2	1.3	3.5	2.3	87

*MSE = $\Sigma(\hat{Y}-\bar{Y})^2/(n-2)$ where $\hat{Y}$ is the estimated batch fecundity from subsamples. MSE, rather than R^2, is used to measure the fitness of the model because R^2 is inappropriate for nonlinear equations.

Precision of Batch Fecundity Estimation and the Numbers of Females

In the previous sections, we have considered the optimum number of ovarian tissue samples of northern anchovy relative to costs and have examined various regression models for biases in expressing the fecundity-fish weight relationship. This analysis indicated that if two or three tissue samples per fish are taken, further precision can be obtained only through increasing the numbers of fish in the sample, and we also showed that for anchovy the simple linear model is the preferred regression model. The objective of this section is to determine the effect of the number of females on the precision of the regression estimates of batch fecundity ($\bar{Y}$) using the linear model. All the historical data for batch fecundity in the northern anchovy (1951-84) were used in this analysis.

The average batch fecundity for the spawning population can be estimated ($\hat{\bar{Y}}_{\bar{\bar{w}}}$) from a regression model where $\bar{\bar{w}}$ is an average of ovary-free female fish weight for the survey. Batch fecundity is based

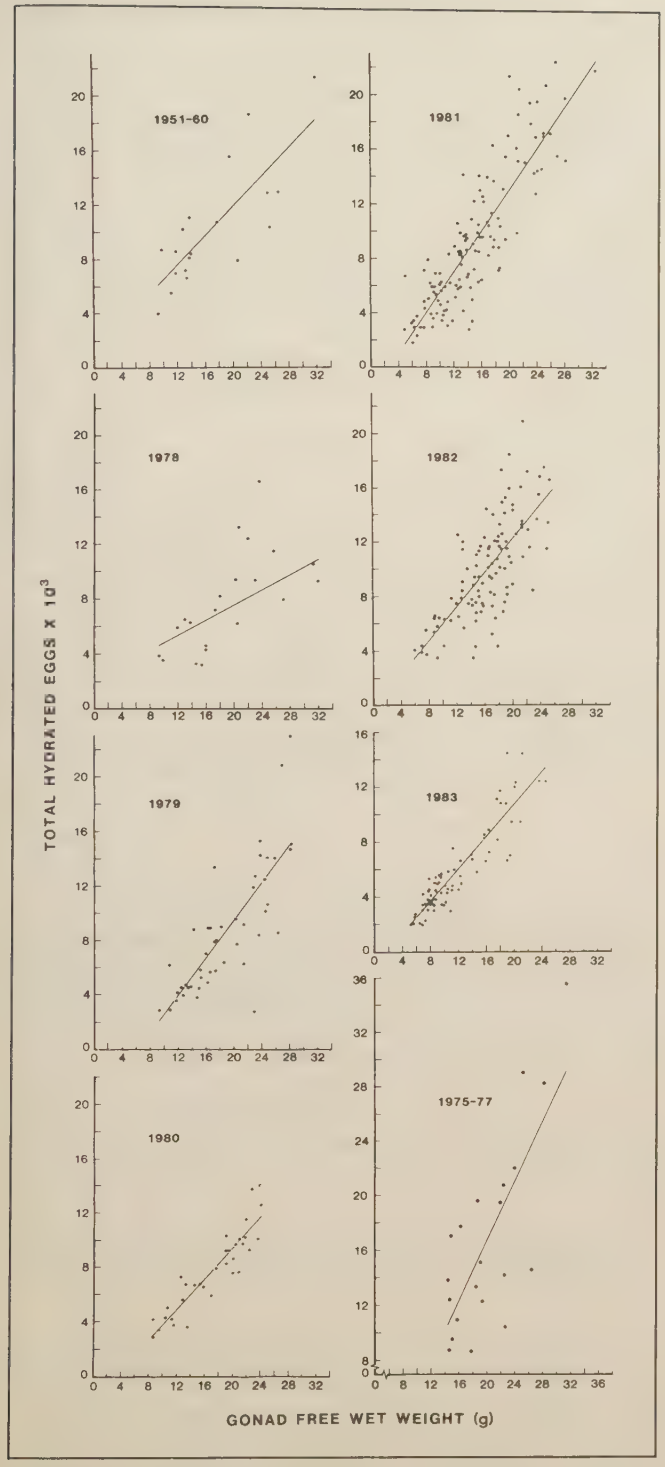

Figure 4.—Relation between batch fecundity of northern anchovy and their weight in formaldehyde solution (less ovary) for various years and subpopulations. All panels except lower right are for central subpopulation; lower right is for northern subpopulation, 1975-77, from Laroche and Richardson (1980). Fecundity estimates for the central population for 1951-60 are from MacGregor (1968) and are estimated using the oocyte frequency distribution method. Equations for lines are given in Table 4 for central subpopulation. For northern subpopulation $\hat{Y} = -5287 + 1098\ w$, $R^2 = 0.55$ (our calculation from Laroche and Richardson 1980).

on ovary-free weight whereas the weight of each female in the survey includes the ovary weight. Thus the mean weight of females ($\hat{w}$) in the survey must be adjusted to ovary-free weight to estimate the batch fecundity for the average female in the population. This is done by multiplying female weight by 0.95, i.e., $\bar{\bar{w}} = 0.95 \cdot \hat{w}$ (Hunter and Macewicz 1980) (Table 4). The average female weight in the fecundity samples ($\bar{w}$) may be different from $\bar{\bar{w}}$ because the fish used in the fecundity estimate are a small subsample of the thousands of mature fish taken in a survey. Hence, $\bar{\bar{w}}$ is preferable for biomass estimation. Generally speaking, $\bar{w}$ and $\bar{\bar{w}}$ are similar although occasionally the difference has been as great as 2 g.

The precision of the regression estimate of batch fecundity is measured by its coefficient of variation, $cv(\tilde{Y}_w)$. The variance of $\tilde{Y}_{w^*}$ for a particular fish weight w^* is equal to

$$\sigma^2 \cdot \left[\frac{1}{n} + \frac{(w^* - \bar{w})^2}{\sum (w - \bar{w})^2} \right] \tag{10}$$

where σ^2 is the variance around the regression line (estimated by s_ξ^2) and w^* is measured without error. To evaluate the effect of numbers of fish on the precision of the regression estimate, we calculated the cv of $\tilde{Y}_{\bar{w}}$ and $\tilde{Y}_{\bar{w}-2}$ for each of the data sets. Four elements affecting the variance of $\tilde{Y}_{w^*}$ are σ^2, n, w^*, and the sums of squares ($\Sigma(w-\bar{w})^2$). Although the variances of fish weight (s_w^2) are quite similar among years, the sum of squares ($\Sigma(w-\bar{w})^2 = (n-1)s_w^2$) are different (Table 4). A small value of $\sigma^2_{\tilde{Y}_{w^*}}$ results from either a large n and/or a wide range of fish weights. Increasing sample size n and/or increasing $\Sigma(w-\bar{w})^2$ reduces $\sigma^2_{\tilde{Y}_{w^*}}$.

The coefficient of variance of $\tilde{Y}_{\bar{\bar{w}}}$ was expressed as a function of the number of fish in the fecundity sample. For this analysis we used two versions of the power function ($cv(\tilde{Y}_w|n) = \alpha \cdot n^\beta$): One in which $\bar{\bar{w}}$ is assumed to be the same as $\bar{w}$ and the other in which $\bar{\bar{w}}$ is equal to $\bar{w} - 2$ [Equations (11) and (12)]. In the latter equation, the cv of $\tilde{Y}_{\bar{w}-2}$ sets the upper bound for the cv of $\tilde{Y}_{\bar{\bar{w}}}$.

$$cv\left(\tilde{Y}_{\bar{w}-2}\,|n\right) = 0.644\, n^{-0.735} \tag{11}$$

$$cv\left(\tilde{Y}_{\bar{\bar{w}}}\,|n\right) = 0.438\, n^{-0.561} \tag{12}$$

For a sample size of 20 fish, the cv of $\tilde{Y}_{\bar{\bar{w}}}$ falls between 0.07 and 0.08, and for a sample size of 60 it ranges between 0.03 and 0.04 [depending upon whether Equation (11) or (12) was used] (Fig. 5). Assuming that the coefficient of variation for the fecundity estimation should be less than 0.10, one should select a sample size that yields a cv of $\tilde{Y}_{\bar{\bar{w}}} = 0.05$ because Equations (11) and (12) assume the fish weight (w) is measured without error; thus, for northern anchovy, a sample size of 50-60 females is adequate. In the biomass estimation, the cv for the fecundity estimation is a function of variances of both $\tilde{Y}_{\bar{\bar{w}}}$ and $\bar{\bar{w}}$. The variance formula for the fecundity estimate including variance of $\bar{\bar{w}}$ is given below.

$$\sigma^2_{\tilde{Y}_w} = \sigma^2_{\left[\bar{Y} + b\,(\bar{\bar{w}} - \bar{w})\right]} = \sigma^2 \left[\frac{1}{n} + \frac{(\bar{\bar{w}} - \bar{w})^2}{\sum (w - \bar{w})^2} \right] + b^2 \sigma^2_{\bar{\bar{w}}} \tag{13}$$

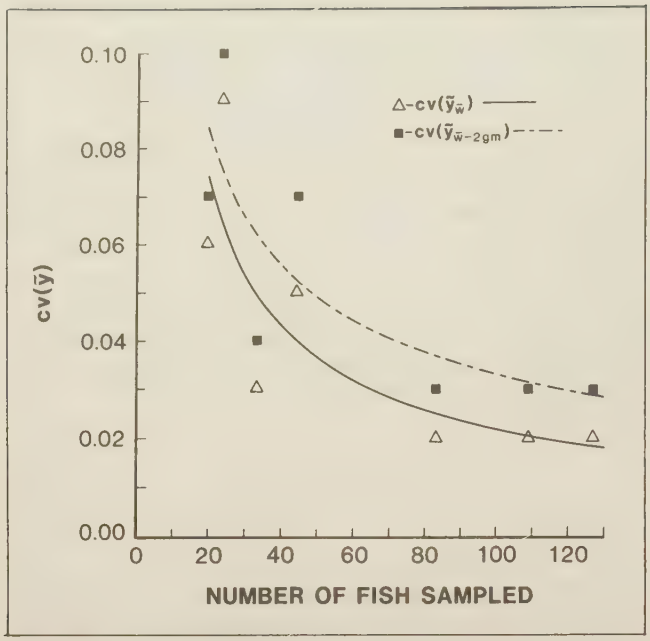

Figure 5.—Coefficient of variation (*cv*) of the regression estimates of batch fecundity for the average female anchovy weight as a function of the number of fish used to estimate fecundity in various years (see Table 4). Solid line is the *cv* for the average weight of females in the fecundity sample, and dashed line is the upper bound of the *cv* for the average female weight in the population.

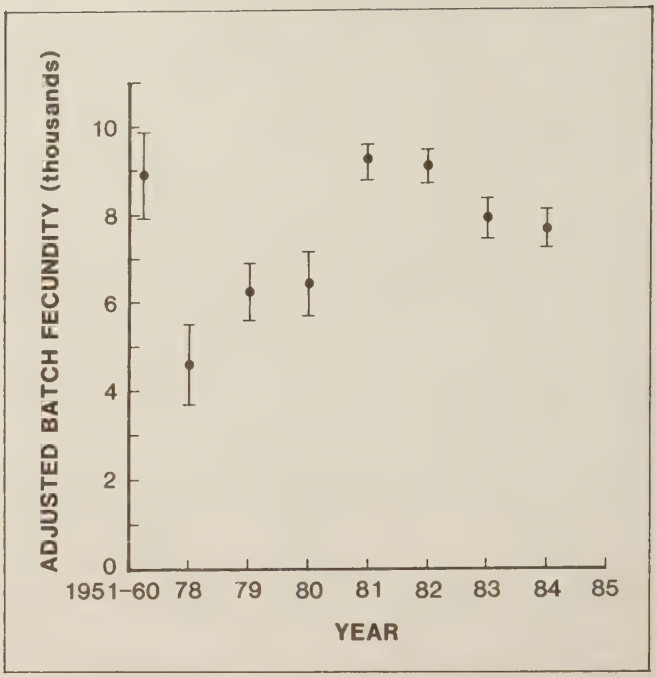

Figure 6.—Adjusted batch fecundity estimates for average female weight equal to 14.95 g, and the 95% confidence interval (represented by vertical bars) by years (see Table 4).

Variation of Batch Fecundity Among Years

The relation between batch fecundity and fish weight has been estimated annually for the egg production biomass estimation of northern anchovy because we believed that the relationship could change from year to year. Otherwise one equation would suffice for all years. To test this assumption, we performed an analysis of covariance to compare batch fecundity per female weight (regression coefficients, *b*) among years. The results show that not all slopes are the same, and have ranged from 279 eggs/g in 1978 to 752 eggs/g in 1981 (Table 4). Moreover, the average batch fecundity for a female of 14.95 g (the mean weight of all females in the fecundity samples when all samples are combined) also differs significantly among years (Fig. 6). The average number of eggs produced per spawning by a standard female of 14.95 g has varied by a factor of 2 over the last 7 yr (1978-84). This does not take into account the variation in average weight of females in the spawning population which also shows significant interannual variation. No doubt exists that batch fecundity of the northern anchovy population varies interannually and that it is necessary to estimate batch fecundity for each biomass estimation. On the other hand, if an average fecundity for all years (1951-84) is necessary, the following equation can be applied: $\hat{Y}_w = -1104 + 614\ w$.

SUMMARY

1. Laboratory methods are described for estimating the batch fecundity of fishes with indeterminate annual fecundity using counts of the number of hydrated eggs within weighed tissue samples of the ovary.

2. In northern anchovy, location of the tissue sample within the ovary has no effect on counts of hydrated eggs. Location of tissue samples may be important in fishes with larger ovaries or possibly in anchovy in the earliest stages of hydration. Thus, position effects must be evaluated for each species and sampling time.

3. The optimum number of tissue samples was 2 or 3 per ovary in northern anchovy, but a higher number may be necessary if position effects exist.

4. To maintain the coefficient of variation for the average fecundity of the population at less than 10% requires a sample of 50 or more females.

5. In northern anchovy, a simple linear regression model was preferable to nonlinear models for expressing the fecundity-female weight relation but this must be evaluated for each population.

6. For the egg production estimates, the average batch fecundity of the population can be calculated from the average weight of the females in the population (based on a weighted sample mean) using batch fecundity-female weight regression model.

7. The batch fecundity of northern anchovy has varied significantly among years (1951-84) indicating the batch fecundity-fish weight relation must be estimated for each egg production estimate.

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ADDENDUM

Since this volume went to press an additional estimate of the biomass of the central subpopulation of northern anchovy was made in 1985. Batch fecundity parameters (listed in Table 4) for 1985 were as follows: n, 85; b, 682; a, −2,036; $s_{y \cdot x}$, 1,936; $\bar{Y}$, 8,490; $\bar{w}$, 15.43; s_w, 4.26; $(w-\bar{w})^2$, 1,524; $\bar{\bar{w}}$, 14.50; $s_{\bar{\bar{w}}}$, 0.32; adjusted mean batch fecundity for 14.95 g fish, 8,156; and standard error of batch fecundity for 14.95 g fish, 211.

Measurement of Spawning Frequency in Multiple Spawning Fishes

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ABSTRACT

Methods for estimating the frequency of spawning in natural fish populations using histological criteria are described using the northern anchovy, *Engraulis mordax*, as an example. Illustrations and descriptions are provided of oocytes in mature ovaries, postovulatory follicles, and atretic follicles. Postovulatory follicles are used to estimate frequency of spawning, and atretic follicles (which may be confused with postovulatory follicles) are useful in estimating size at first maturity. Postovulatory follicles of four common commercial fishes, *Sardinops sagax*, *Merluccius gayi*, *Scomber japonicus*, and *Euthynnus lineatus*, are also described and illustrated. Methods for estimating spawning frequency other than ovarian histology are also discussed, as is testing of the assumption of determinate annual fecundity in multiple spawning fishes.

INTRODUCTION

The egg production method of biomass estimation requires an estimate of spawning frequency. This requirement exists because the method was developed for fishes, such as the northern anchovy, which have indeterminate annual fecundity. In such, the standing stock of advanced oocytes gives no indication of annual fecundity because new spawning batches are recruited from small, unyolked oocytes during the spawning season. Thus, the rate of egg production can be calculated only from estimates of spawning frequency and batch fecundity made during a survey period. On the other hand, in some multiple spawning boreal fishes, including haddock, *Melanogrammus aeglefinus*, and whiting, *Merlangius merlangus* (Hislop 1975; Hislop et al. 1978; Hislop[1]), and other fishes (Yamamoto 1956), the stock of oocytes destined to be spawned in a season is identifiable at the beginning of the season. In such fishes, annual fecundity may be considered to be determinate even though the fishes may spawn repeatedly during the season and the standing stock of advanced oocytes at the beginning of the season is considered to be equivalent to the annual fecundity. Hence, estimates of spawning frequency are unnecessary in fishes with determinate annual fecundity as long as the biomass survey includes the entire spawning period and nearly all of the stock of advanced oocytes are actually spawned. In some cases, not all the standing stock of oocytes are spawned and, consequently, potential annual fecundity is not realized during the spawning season. Documentation of the existence of determinate annual fecundity, and of its total utilization during the spawning season, is essential if the standing stock of oocytes is used to estimate spawning biomass. This is rarely done satisfactorily, and in a number of cases we believe the presumption of a seasonally determined fecundity is probably false which may lead to serious errors in biomass estimation. Estimation of the frequency of spawning and batch fecundity is one way to test the assumption of determinate annual fecundity.

The objective of this paper is to describe the methods for estimating frequency of spawning in natural fish populations. We begin with a brief discussion of histological methods, followed by a general description of the oocytes in an active ovary of northern anchovy, *Engraulis mordax*. We then describe the estimation of spawning frequency using aged postovulatory follicles (employed in the current method for biomass estimation of northern anchovy) and briefly discuss other methods. In the second section of the paper we discuss ovarian atresia, because histologists must be able to distinguish postovulatory follicles from atretic follicles, and knowledge of atresia is also necessary to separate postspawning females from immature ones. In the final sections we discuss tests of the assumption of determinate fecundity and future applications of histological classification of ovaries.

Most of the descriptions and all of the data in this paper are for the northern anchovy. Nevertheless we have tried to bring out important differences that may exist among species and to mention methodologies other than the ones we use for northern anchovy.

HISTOLOGICAL METHODS

All the techniques discussed in this paper involve, at least to some extent, histological examination of fish ovaries. In this section we briefly discuss the histological techniques used and comment on the

[1]John R. G. Hislop, DAFS Marine Lab., P.O. Box 101, Victoria Road, Aberdeen AB9 8DB, Scotland, pers. commun. 25 Oct. 1983.

procedures and time required to use histological classification for fish population work.

Histological preparation of tissues

Almost all northern anchovy ovaries histologically examined for postovulatory follicles were fixed in 10% neutral buffered Formalin (NBF), embedded in Paraplast, and 6-μm serial sectioned slides were made and stained by Harris' hematoxylin followed by Eosin counter stain. This procedure gave good results, and specific steps can be found in Luna (1968), Preece (1965), or most any histotechnique handbook. It is also possible to use resin embedding, sectioning on a rotary microtome, and Lee's Methylene blue-basic fuchsin staining (JB-4 Embedding Kit, Data Sheet No. 123, Polysciences, Inc., 1982). This technique may be preferable for small ovaries or small pieces of ovaries, although it is more difficult to get many serial sections and may not be practical for larger ovaries.

In addition to the instructions given in standard histotechnique texts, we found the following procedures and precautions to be useful in preparation of Hematoxylin and Eosin (H&E) sections of northern anchovy ovaries.

Fixation—Bouin's fixative gave better results than 10% NBF, but it required transferring ovaries into 70% ethanol within 1-2 d or the ovaries become hard and brittle. Transferring is possible in the laboratory but at sea it is often impractical. It is important to fix ovaries quickly and thoroughly, otherwise lysis or poor differentiation may occur. We insured proper exposure to fixative by slitting the abdomen of fish before fixing. In larger fish it is best to remove the whole ovary from the fish and preserve it separately. It is even better to cut a 1-cm section from the midsection and preserve it in ample fixative. Initial freezing of whole fish or ovaries is not recommended; fixation after freezing results in poor-quality H&E sections in which postovulatory follicles cannot be consistently identified, thus producing substantial errors in the estimation of spawning frequency. In addition, the process of thawing may cause lysis of hydrated oocytes.

Infiltration—Hardening of ovaries may occur if time spent in xylene or toluene is not kept at a minimum or if temperature of paraffin is not kept below 60°C.

Sectioning—It is necessary to have 10-20 good serial sections per slide for histological analyses. Cold (4°C) blocks and knives usually improve sectioning. If shattering occurs, it is possible to soak blocks (prefaced-off) in ice water for a short time (1-15 min) to improve sectioning with little or no shattering.

Mounting—Wiping slides with a very fine coat of Mayer's Albumen Affixative and drying mounted slides are often necessary to keep sections attached to slides. By mounting as many sections as possible on a slide, it may be possible later to microscopically trace questionable structures.

Staining—The standard procedure for Harris' Hematoxylin and Eosin staining is quick and easy, and results in good staining of postovulatory follicles. Initially a trial run is necessary to find out the optimal amount of time spent in each stain. Other stains, i.e., Mason's Trichrome, periodic acid-Schiff reagent, or Heidenhain's iron hematoxylin, can also be used except they often require more time or more elaborate techniques. These stains may be preferable for study of structures other than postovulatory follicles, for example, nuclear changes in oogenesis.

Rates of Processing and Classification

The production of slides depends largely upon the capacity of the automatic tissue infiltrator and the number of histotechnicians. In small research laboratories (infiltrator capacity = 30 samples per batch) production of 100-150 H&E slides (one ovary per slide) per week for one person is close to the maximum rate of production. Thus, for production work it is preferable to use commercial histological laboratories where H&E slides can be produced more quickly at a cost of (in 1983) about $4.00/slide.

The time required to classify each slide of ovarian tissue (about 10 sections per slide) depends upon the amount of detail recorded. If only spawning frequency is estimated (ageing postovulatory follicles and identification of hydrated oocytes), one person can classify about 1,000 slides in 25 d (8 h/d). Two to three times as much time is required if all other significant histological characteristics of the ovary are recorded, including the presence and abundance of alpha through delta stages of atretic yolked and unyolked oocytes and the relative abundance of oocyte classes. We have employed this detailed system over the years to provide a complete record of the condition of the ovary. However, our analysis indicates that the system can be simplified by identifying only hydrated oocytes, two age classes of postovulatory follicles, and by classifying ovaries into three atretic states (defined in a subsequent section). This reduces classification time per ovary to nearly the equivalent of that required to estimate only spawning frequency, yet the ability to identify major changes in atretic condition, and to separate postspawning females from immature females, is still maintained.

HISTOLOGICAL CHARACTERISTICS OF OOCYTES

The northern anchovy is a multiple spawning fish (Hunter and Goldberg 1980) with asynchronous oocyte development, i.e., oocytes in many stages of development occur simultaneously in reproductively active ovaries (Wallace and Selman 1981). During the spawning season, oocyte development is a continuous process involving all stages of oocytes, with a new spawning batch maturing every week to 10 days in peak spawning months (Hunter and Leong 1981). The average female anchovy spawns at least 20 times per yr, yet within the ovary only 1-3 potential spawning batches of yolked oocytes exist and fewer than 10 potential batches exist when all oocytes >0.1 mm are included (Hunter and Leong 1981). Oocytes ≤ 0.1 mm constitute the reservoir of oocytes that are present in ovaries year-round in inactive as well as active ovaries. Little or no atresia occurs among oocytes in this size range. Thus, in anchovy spawning, batches must be recruited from the reservoir of oocytes ≤ 0.1 mm to account for the observed frequency of spawning. This means the fecundity of northern anchovy is clearly seasonally indeterminate.

Oocyte development and maturation in teleosts, reviewed recently by Wallace and Selman (1981), has been frequently subdivided into many stages (Yamamoto 1956; Lambert 1970b), but a simpler histological classification system seems appropriate for the purpose of this manual. We have combined the stages of past authors into four oocyte classes (unyolked, partially yolked, yolked, and hydrated), and we describe below for the northern anchovy the histological characteristics of each class.

Unyolked Oocytes

This class includes all oocytes without yolk that are between about 0.04 and 0.35 mm (Fig. 1a, b). Oocytes <0.04 mm are excluded because they consist mostly of "oogonial nests," have no true follicle layer, and appear not to undergo degeneration (Fig. 1b). The smaller oocytes within this class (0.04-0.15 mm) are spherical, have a large nucleus, and have cytoplasms that are narrow, homogeneous, and very densely stained with hematoxylin (Fig. 1b). A very thin, single layer of elongated, spindle-like cells (the beginning of the granulosa layer) surrounds these small oocytes. The large oocytes in this class are oval; their cytoplasm stains faintly with hematoxylin and has a cloudy, mottled appearance (Fig. 1d). The oval nucleus of these oocytes contains several nucleoli and is surrounded by a granular perinuclear zone. In these larger oocytes a thin, definite, hyaline membrane which has a faint eosinophilic stain (percursor of the zona radiata) appears between the oocyte and the growing follicle. The follicle consists of a narrow, single, inner layer of cuboidal granulosa cells and a single outer layer of flat elongated thecal cells with some blood capillaries. The larger oocytes also may have some small vesicles in the periphery of the cytoplasm. These vesicles are at times difficult to distinguish and they seem to disappear in yolked oocytes. No oil vacuoles exist, as northern anchovy eggs do not contain oil droplets.

Partially Yolked Oocytes

Oocytes in this class are in the early stages of yolk deposition (vitellogenesis) and range in size from 0.3 to 0.5 mm (major axis) (Fig. 1d, g). The class includes oocytes in the initial stage of yolk deposition up to and including those in which yolk granules or spherules extend 3/4 of the distance from the periphery to the perinuclear zone. Yolk deposition starts at the periphery of the oocyte cytoplasm as small eosinophilic staining granules and then subsequently spreads internally until it nearly reaches the finely granular perinuclear zone. Usually by this time the granules have become small spherules. The oval-shaped nucleus of oocytes in this class contains several nucleoli. At the time yolk appears in the oocyte, delicate striations appear on the hyaline membrane between the oocyte and follicle layer and it is henceforth referred to as the zona radiata. As maturation proceeds, the follicle layer becomes wider due to an increase in the width and proliferation of the granulosa cells. The thecal cells do not increase in size but remain elongated, flat cells with occasional blood capillaries, and form a thin outer covering to the follicle. The thecal cells do not change until hydration, when they become even flatter and have a stringy appearance.

Yolked Oocytes

Oocytes in this class range from 0.45 to 0.80 mm (major axis) and all contain yolk spherules or globules throughout the region between the periphery of the oocyte and the perinuclear zone (Fig. 1c, d). As vitellogenesis continues, the yolk varies from spherules in the smaller oocytes to large globules in the larger ones. Just prior to spawning (<24 h) the globules fuse to form yolk plates (Fig. 1h). Such oocytes are excluded from this class, since this characteristic is diagnostic of the last (hydrated) class of oocytes. The nucleus of oocytes in the yolked class is oval with numerous nucleoli and is centrally located until just before hydration, when it migrates to the animal pole. The granulosa cells have a wide rectangular shape in cross section and a large oval nucleus; their walls are clearly evident in sagittal section where they form polyhedrons. The zona radiata is a wide, striated, eosinophilic band until hydration, when it stretches thin and the striations seem to disappear.

Hydrated Oocytes

These oocytes range in size from 0.75 to 1.2 mm (major axis) (Fig. 1g, h). Hydration (rapid uptake of fluid by the follicle; Fulton 1898) begins at about the time the nucleus has completed its migration to the animal pole (Fig. 1e, f) and yolk globules have begun to fuse, forming yolk plates. The nucleus of a hydrated oocyte is not visible because after the nucleus has arrived at the animal pole the nuclear membrane disintegrates, dispersing its contents into the cytoplasm. During hydration, all yolk globules fuse into plates and the oocyte expands greatly, stretching the granulosa and thecal cell layers. At this time the granulosa cells in cross section appear as long, thin rectangles, the thecal cells are extremely flat and have a string-like appearance, and the zona radiata is very thin and lacks striations. Hydrated oocytes are the most ephemeral of all oocyte classes since this stage lasts for less than a day, whereas the other stages are always present in reproductively active anchovy ovaries. Migratory nuclei may be seen as early as 24 h before ovulation, but hydrated oocytes in which all globules are fused to form yolk plates do not occur earlier than 12 h before spawning. We have observed no atresia in hydrated oocytes; apparently, in northern anchovy, nearly all hydrated oocytes are ovulated.

The hydrated oocyte stage in northern anchovy begins when the nuclear membrane disintegrates and ends at ovulation. As this was an arbitrary decision, the duration of this stage could be increased by including the period of nuclear migration. This broader definition of the hydrated stage might be useful when sampling occurs before complete hydration or if hydration occurs very rapidly, as might be the case in tropical fishes.

ESTIMATION OF SPAWNING FREQUENCY

Postovulatory Follicle Method

In northern anchovy, each hydrated oocyte is surrounded by a thinly stretched follicle of an inner, epithelial layer of granulosa cells and a single, outer connective tissue layer of thecal cells with some blood capillaries. At ovulation, the fully hydrated oocytes are released from their encompassing follicles. The follicle does not fragment and pass out of the ovary with the hydrated oocyte but retains its integrity. The follicle collapses away from the opening formed for the release of the hydrated oocyte into the lumen and remains in the ovary as an evacuated follicle, or postovulatory follicle. Spawning occurs simultaneously with ovulation or in <60 min after ovulation. Initially, the postovulatory follicle is a distinct structure, but it rapidly deteriorates and is resorbed. In northern anchovy, by 48 h after ovulation postovulatory follicles can no longer be accurately discriminated from the intermediate stages of atretic oocytes.

To use postovulatory follicles for estimation of spawning frequency, it is necessary to divide the deterioration and resorptive processes of the follicle into a series of distinct histological stages, each with an assigned age (time from spawning). This requires a series of ovary samples taken at regular intervals from the time of spawning and can be accomplished by spawning fish in the laboratory (Leong 1971)

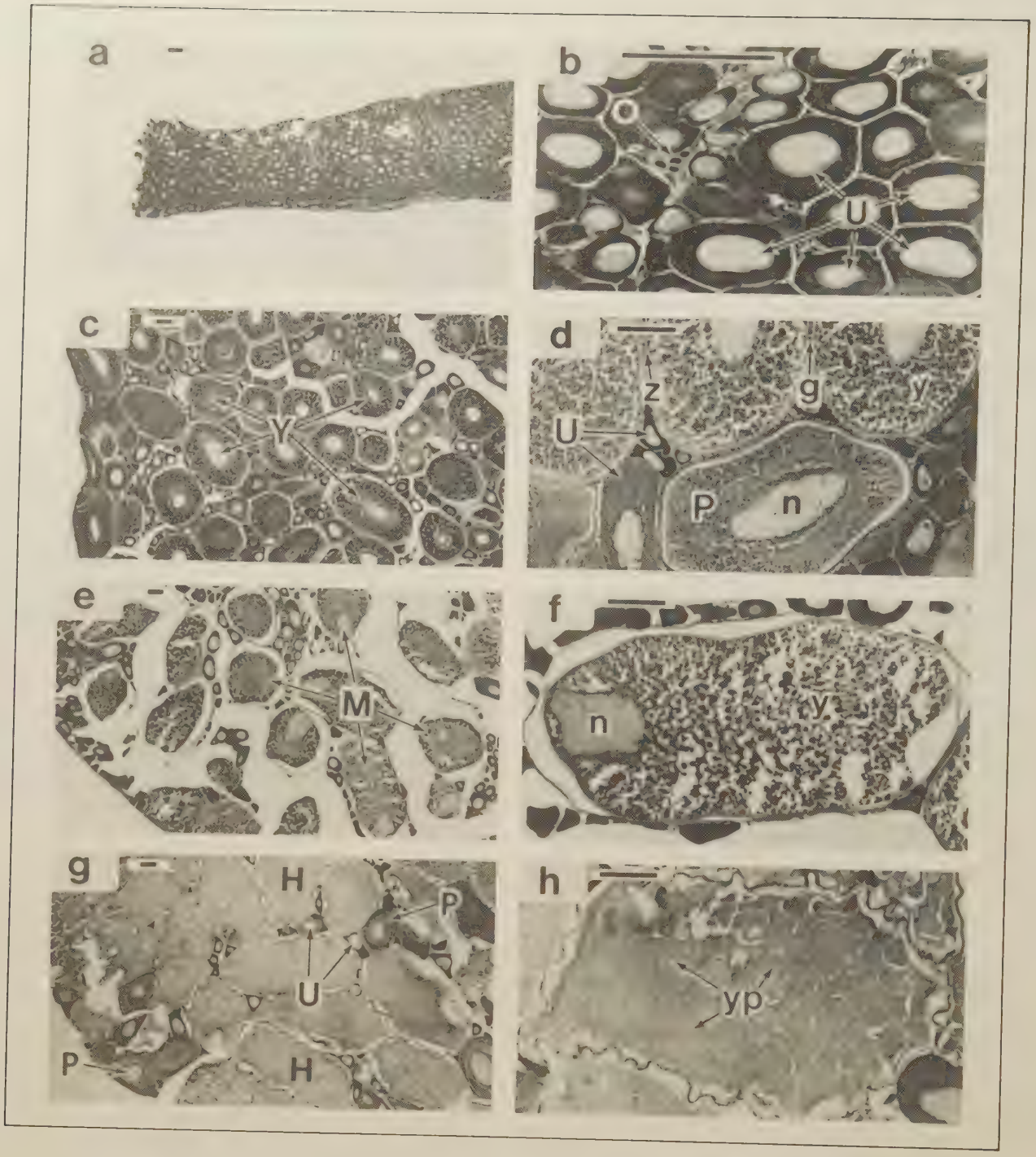

Figure 1.—Development of northern anchovy ovary at various magnifications. Stain = H&E; bar = 0.1 mm.

a) Immature ovary consisting of unyolked oocytes and no atresia.

b) Enlargement of (a) showing small, spherical, unyolked oocytes (U) with a large central nucleus and "oogonium nests" (o).

c) Normal, mature ovary with many fully yolked oocytes (Y).

d) All stages of oocytes: unyolked (U), partially yolked (P), and yolked (Y), are present in normal mature ovaries. (g = granulosa epithelial cell layer; z = zona radiata; n = nucleus; y = yolk globules.)

e) Prespawning ovary showing oocytes in the migratory nucleus stage (M).

f) Enlargement of a migratory nucleus oocyte (M). (n = nucleus; y = yolk globules.)

g) Imminent (<12 h) spawning ovary with hydrated oocytes (H) still within their follicle layers. (U = unyolked; P = partially yolked.)

h) Enlargement of a hydrated oocyte. Note that the yolk globules have fused into yolk plates (yp), and there is no prominent nucleus due to disintegration of the nuclear membrane.

or by sampling spawning schools over a 24-h period in the sea (Goldberg et al. 1984; Alheit et al. 1984). The latter approach requires estimating the average time of spawning for the population and has somewhat greater uncertainties than the preferable laboratory approach. At moderate temperatures (~16°C) we recommend taking samples of at least 10 females with postovulatory follicles at 4-h intervals for 3-4 d. At higher spawning temperatures, e.g., 28-30°C, sampling at 1-2 h intervals might be required. Once the classification criteria and stages are defined, it is necessary to conduct a blind classification of ovaries that include females with all stages of postovulatory follicles as well as those without these structures. The classification system we employ for northern anchovy is described below, and the results of the original blind classification are given in Hunter and Goldberg (1980), although our level of accuracy has considerably improved since that initial test.

Age "0 Day" postovulatory follicles in anchovy—Included in age 0-day postovulatory follicles are those new, remnant, evacuated follicles that are from 0 to 23 h old. In our surveys, however, we include in this age those postovulatory follicles about 0 to 5 h old since we trawl for northern anchovy only during the nightly spawning period (1800-0300 h). In laboratory-spawned females sacrificed 0-4 h after spawning, the new postovulatory follicles show no signs of degeneration but they may undergo some structural changes. At ovulation, while the follicle is collapsing, the follicle cell layers, appearing cord-like, form loose folds or loops. The granulosa cells, which had been extensively stretched during hydration, appear elongated and narrow with a large prominent nucleus in the center (Fig. 2a); the thinly stretched thecal layer thickens and becomes more noticeable. After spawning, the fully collapsed postovulatory follicle is a much more tightly folded or looped structure. It is relatively large, irregular in shape, with an irregular lumen which frequently contains eosinophilic granules of uncertain origin. The granulosa cells of this new postovulatory follicle (age-0 day) are characteristically columnar or cuboidal and in some cases have hypertrophied slightly; these cells are arranged orderly along the edge of the lumen with their cell walls usually evident and possessing prominent nuclei. The nucleus of the granulosa cells may be located at either the apex or base of the cell. In field-caught fish, the follicles with apical nuclei appear to occur in fish taken near the time of spawning and those having basal nuclei somewhat later (Fig. 2b, c). After spawning, the thecal cell layer is more clearly defined, adheres closely to the granulosa layer, and contains blood capillaries.

We use the above characteristics of the follicle plus no signs of follicle degeneration as the diagnostic characteristics of postovulatory follicles of age 0-day. Our surveys occur only at about the time of spawning, about 1800 to 0300 h, an insufficient period for significant follicle degeneration to occur. If sampling times were extended 6-8 h or longer after the peak hour of spawning, early degenerative follicle characteristics would have to be included as a characteristic of age 0-day postovulatory follicles. Degeneration appears to start 6-8 h after spawning in northern anchovy. The first sign of degeneration is the presence of a few pycnotic nuclei in the granulosa cells, followed shortly by the migration of a few lymphocytes into the postovulatory follicle; finally a few vacuoles appear in the granulosa layer. In northern anchovy, all of these events occur before the postovulatory follicle is 12 h old.

Age "1 Day" postovulatory follicles in anchovy—Degeneration is pronounced in postovulatory follicles examined about 24 h after spawning. The characters we use to classify postovulatory follicles as age 1-day are those that appear between 19 and 28 h after spawning, since our surveys occur only near the time of spawning. We describe follicles of age 19-28 h and subsequently discuss identification of older follicles.

By 19-28 h after spawning, the regressing postovulatory follicle is greatly shrunken and has fewer folds, hence a less irregular form than a new postovulatory follicle. The lumen, which is much reduced, may contain some granular material, although not as much as occurs in the lumen of 0-day postovulatory follicles. The granulosa cells of the follicle no longer have the orderly alignment characteristics of age 0-day postovulatory follicles, although some pattern in arrangement of cells can still be seen. Some of the nuclei of the granulosa cells are pycnotic, vacuoles are common, and only few of the cell walls are intact. The underlying layer of thecal cells is present, although less distinct, than in an age 0-day postovulatory follicle (Fig. 3).

Postovulatory follicles older than 28 h in anchovy—Classification beyond 28 h becomes difficult as the follicle continues to degenerate, and by 48 h it may easily be confused with the beta stage of atretic follicles (see section on atresia). A postovulatory follicle 48 h old is one-half to one-fourth the size of 24-h-old follicles, the lumen is much reduced or absent, and no eosinophilic granules are present. Cell walls are absent in the remaining granulosa layer tissue, and a few vacuoles or pycnotic nuclei may be seen. The theca is present but is often indistinct as it becomes incorporated into the ovarian connective tissue stroma. The number of postovulatory follicles in the ovary appears to be reduced by 48 h after spawning. Fewer follicles may be present because they were resorbed or, alternatively, fewer may be seen per section because of the growth of the larger oocytes. We believe that by 3-4 d after spawning all postovulatory follicles have been resorbed, although they might produce a structure that is indistinguishable from delta-stage atretic follicles. However, delta-stage atretic follicles are far less numerous in the ovary than new postovulatory follicles.

Postovulatory follicles in other fishes—The structure of ovaries among teleosts is relatively similar to anchovy especially in species that spawn unadorned pelagic eggs. As far as we can ascertain, the postovulatory follicles of such fishes differ only in minor details from the descriptions we have provided for the northern anchovy. The postovulatory follicles of the Peruvian anchovy, *Engraulis ringens*, is identical in all respects to that of the northern anchovy, regardless of follicle age, and hence will not be discussed further. We illustrate and briefly describe postovulatory follicles of four other species to indicate the variation in these structures among common commercial fishes that spawn unadorned pelagic eggs of about 1 mm diam. These fishes include Pacific sardine, *Sardinops sagax*, Chilean hake, *Merluccius gayi*, chub mackerel, *Scomber japonicus*, and black skipjack, *Euthynnus lineatus*.

The new postovulatory follicle (near the time of spawning) of these four species has the following characteristics in common with northern anchovy: a very convoluted shape with many folds or loops; a lumen containing some granular or particulate material; a definite granulosa epithelial cell layer lining the lumen; linearly arranged granulosa cells of cuboidal or columnar shape which contain a prominent nucleus; a definite thecal connective tissue layer with blood capillaries; and, most importantly, no degeneration of the follicle. Various minor differences from anchovy in the appearance of a new postovulatory follicle are mentioned below. The new postovulatory follicles of chub mackerel seem to differ from anchovy only in the presence of fewer granules in the lumen just after spawning (Fig. 4a, b). In the Chilean hake, the granulosa layer at the time of spawn-

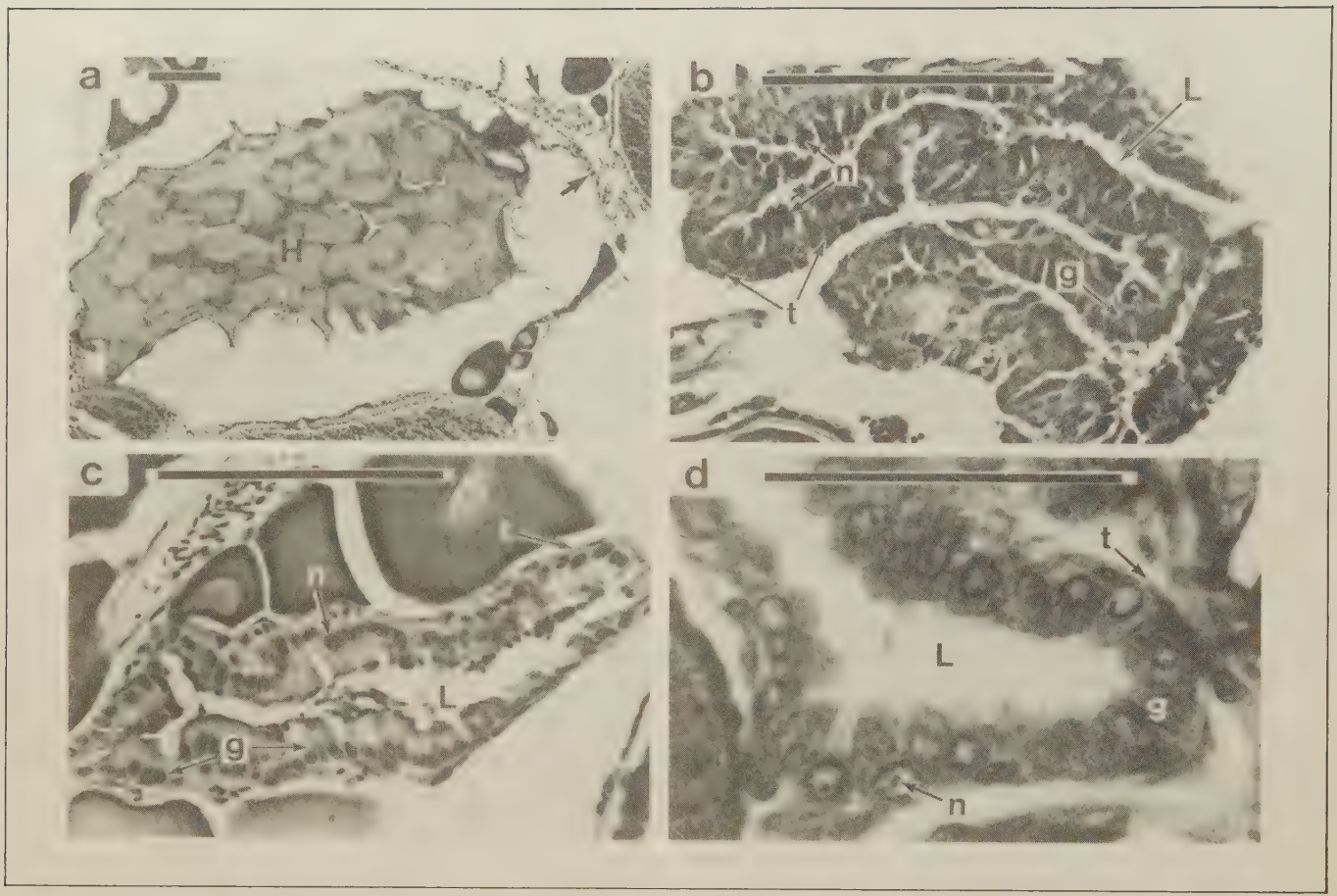

Figure 2.—Postovulatory follicles of age 0-day; elapsed time from spawning about 0-6 h. In a, b, and c, bar = 0.1 mm; in d, bar = 0.05 mm; g = granulosa epithelial cell layer; t = thecal connective cell layer; and L = lumen of follicle.

a) At ovulation the postovulatory follicle (arrow) collapses away from the hydrated oocyte (H).

b) Newly collapsed follicle with nuclei (n) at the apex of the granulosa cells (g). Elapsed time from ovulation <1 h.

c) Follicle with the nuclei (n) at the base of the granulosa cells (g). Elapsed time from spawning is 1-6 h.

d) Enlargement of follicle age 0-day (from Hunter and Goldberg 1980).

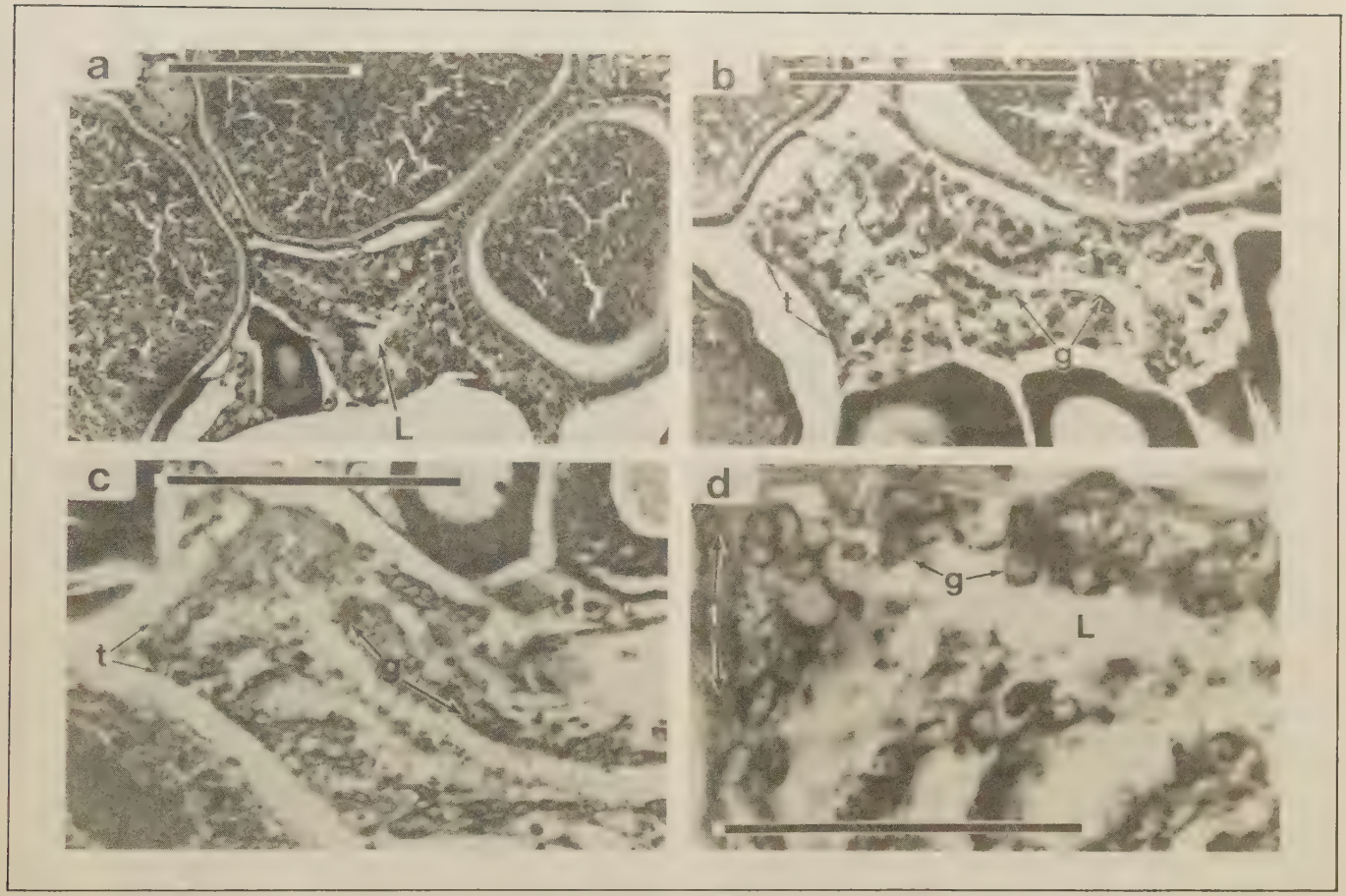

Figure 3.—Postovulatory follicles of age 1-day; elapsed time from spawning about 24 h. Degeneration of the follicle is pronounced by 24 h. In a, b, and c, bar = 0.1 mm; in d, bar = 0.05 mm; g = granulosa epithelial cell layer; t = thecal connective cell layer; L = lumen of follicle.

a, b) Follicles from sea-caught anchovies; the next batch of yolked oocytes (Y) is evident.

c, d) Follicle from anchovy spawned in the laboratory (from Hunter and Goldberg 1980).

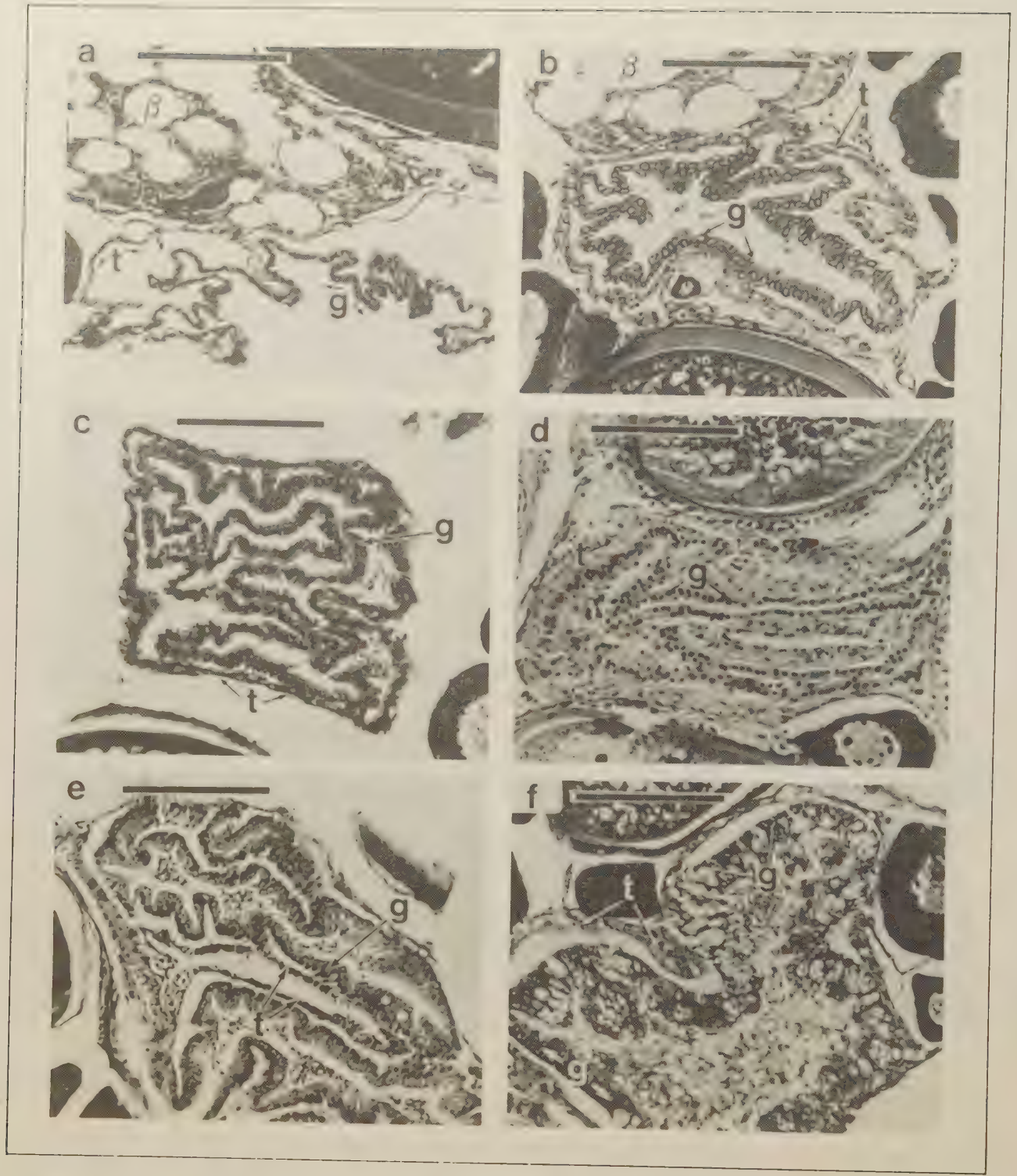

Figure 4.—New postovulatory follicles of four species of marine fishes. Bars = 0.1 mm; β = beta atresia; g = granulosa epithelial cell layer; t = thecal connective cell layer.

a) Chub mackerel, *Scomber japonicus*, at ovulation, and

b) 1 h after spawning (laboratory-induced with hormones). In both sections (a) and (b), a single beta-stage atretic follicle (β) is present; in (a), the atretic follicle contains 12-13 vacuolated areas which may be remnants of the oil droplet.

c) Chilean hake, *Merluccius gayi*. Histological material from S. R. Goldberg, Whittier College, Whittier, CA.

d) Black skipjack, *Euthynnus lineatus*, at the time of spawning (hydrated oocytes were in lumen of the ovary). Histological material from K. Schaefer, IATTC, La Jolla, CA.

e) Pacific sardine, *Sardinops sagax*, from Peru after spawning at sea. Histological material from S. R. Goldberg, Whittier College, Whittier, CA.

f) At ovulation (laboratory-induced with hormones).

ing appears to be coiled up inside a less irregular thecal layer covering, and the lumen is less distinct than anchovy and contains many fewer granules (Fig. 4c). The follicles of black skipjack differ from anchovy only by the presence of a thicker thecal layer that adheres to the granulosa cells (Fig. 4d). The only differences between sardine (Goldberg et al. 1984) and anchovy in the appearance of new postovulatory follicles are the slightly longer columnar granulosa cells, the occurrence of more particles in the lumen, and a few vacuoles in the granulosa cells of the sardine (Fig. 4e). During ovulation the sardine follicle may have quite a different appearance from the anchovy as the granulosa layer contains numerous vacuoles and the cells are more strongly hypertrophic (Fig. 4f). These differences in the appearance of new postovulatory follicles seem trivial relative to their overall similarities, indicating that the new postovulatory follicles of most fishes should be easy to identify using the general characteristics we have described.

We now consider the characteristics of degenerating postovulatory follicles in these four species and how they compare to the descriptions for the northern anchovy. The four species have the following characteristics in common with anchovy: an irregular shape that is smaller and much less convoluted than a new postovulatory follicle; the presence of a lumen, although the size may be greatly reduced; degeneration of granulosa cells (pycnotic nuclei, few cell walls, vacuoles usually present, and lack of alignment of nuclei); and an identifiable thecal layer. The degenerating postovulatory follicle (about 24 h old) of chub mackerel differs from the anchovy because a few red blood cells may occur among the granulosa cells and in the lumen and because very few vacuoles occur in the granulosa layer (Fig. 5a). Bara (1960) noticed red blood cells in the lumen of a degenerating postovulatory follicle of the Atlantic mackerel, *Scomber scombrus*, indicating this may be a common characteristic of *Scomber*. In the degenerating postovulatory follicles of the Chilean hake, some of the pycnotic granulosa cells appeared to have been sloughed into the lumen, a characteristic not seen in anchovy ovaries, and the granulosa cells contained very few vacuoles relative to the anchovy (Fig. 5b). In addition, the thecal layer in the hake now appears to be in closer contact with the remaining granulosa layer than it is in a new postovulatory follicle. The degenerating follicles in black skipjack differ from anchovy by the presence of a much thicker thecal layer surrounding the granulosa layer (Fig. 5c). The degenerating follicle of the sardine (age about 24 h) differs from the anchovy and from a new sardine postovulatory follicle by having a considerably thicker thecal layer and by the separation of the granulosa layer from the thecal layer. In addition, the degenerating granulosa cells of the sardine may be enlarged slightly and may be sloughed into the lumen (Fig. 5d). These differences among degenerating postovulatory follicles indicate minor differences in oocyte structure and perhaps differences in the pattern of resorption among species. However, our descriptions for northern anchovy can be used as a general guide for identification of postovulatory follicles in these and presumably other species. After identification of postovulatory follicles, it is best to age them by laboratory experiments or round-the-clock sea samples (see previous section).

A cautionary note on ageing postovulatory follicles—The persistence of postovulatory follicles in the ovary of the northern anchovy and the Peruvian anchovy is the same, and it appears that Pacific sardine may have a similar duration. These species spawn at moderate temperatures, 13-19°C. In tropical species that spawn at high temperatures, spawning frequency, resorption of postovulatory follicles, and ovarian maturation all may be accelerated. For example, the dragonet, *Callionymus enneactis*, spawns daily at 28-30°C, and postovulatory follicles are not seen 15 h after spawning and were only clearly distinguishable up to 3 h after spawning (Takita et al. 1983). Clearly in such a rapidly maturing ovary, postovulatory follicles would be highly transitory in nature. Thus, the duration of postovulatory stages must be newly estimated for each species, and an assumption that the duration of these stages in a new species is similar to northern anchovy is highly speculative.

Anatomical Maturity Scales and the Gonosomatic Index

Systems for grading ovaries according to macroscopic characteristics have long been used in fishery research; they include the Hjort scale (Bowers and Holliday 1961) and numerous others (see, for example, Holden and Raitt 1974; Macer 1974; Foucher and Beamish 1977; and Robb 1982). Typically, these systems include an immature stage, several stages for maturing ovaries, one for active ovaries, 1-2 stages for hydrated ovaries ("ripe" or "running ripe"), and various stages for "spent" ovaries. At present only the hydrated oocyte stage or stages have sufficiently distinct macroscopic characteristics (large ovary size, large hyaline oocytes) to be of value for estimating spawning frequency.

The terminology used in maturity scales to identify "spent" ovaries is confusing, as "spent" may refer to ovaries with new postovulatory follicles or to atretic postspawning ovaries. Macer (1974) used the terms "spent" and "partially spent" to make this distinction. At present the anatomical stages that seem to be equivalent to postovulatory ovaries are too ambiguous to be used to estimate spawning frequency. It might be possible using a laboratory calibration to identify some macroscopic characteristics that could be used to identify postovulatory ovaries for a short time after spawning. On the other hand, hydrated ovaries and postovulatory ovaries (<24 h old) have similar sampling biases. Thus, the need to develop such anatomical criteria is not great unless postovulatory ovaries could be detected macroscopically 24 h after spawning, which seems unlikely.

The gonosomatic index (GSI)—the ratio of ovary weight divided by fish weight or the equivalent (Davies 1956)—can be used to detect hydrated ovaries since the wet weight of hydrated ovaries is two to four or more times that of other maturity stages. However, the GSI has the inherent problem that dividing by an expression of body size usually does not compensate completely ("normalize") for the effects of fish size (Davies 1956; Vlaming et al. 1982). For the same reproductive state, small fish usually have a lower GSI than do larger fish, and this effect increases with maturation of the ovary (Vlaming et al. 1982; Hunter and Goldberg 1980). In other words, ovary weight increases faster with fish length than does somatic weight. The assumption underlying the GSI is that ovary weight/fish weight relation has the same slope for different maturity stages; this is clearly not the case. Thus for accurate detection of hydrated ovaries, regression analysis or other techniques are probably preferable to the simple ratio. Gonad weight has the same ambiguities as do the maturity scales when used to detect other maturity stages. Postovulatory ovaries differ little in weight from the earlier stages of postspawning (atretic) ovaries.

In summary, the traditional methodologies for assessment of reproductive condition other than histology (anatomical maturity scales, and GSI) can be used to identify females with hydrated ovaries. The incidence of such females with hydrated ovaries might be used to provide at least a crude index of spawning frequency (see

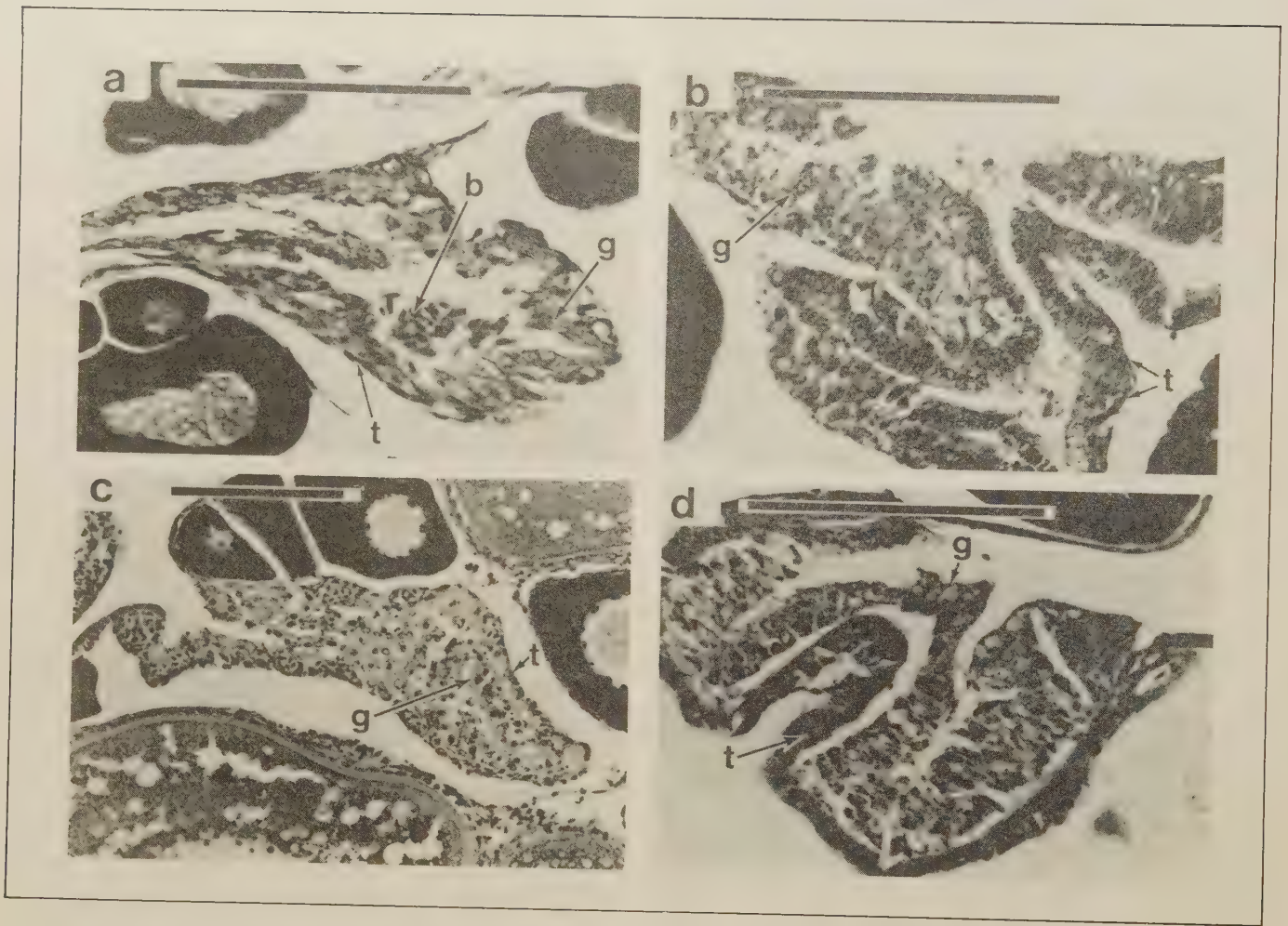

Figure 5.—Degenerating postovulatory follicles in four marine fishes. Bars = 0.1 mm; g = granulosa epithelial cell layer; t = thecal connective cell layer; b = red blood cells.

a) Chub mackerel 24 h after spawning (laboratory-induced with hormones).

b) Chilean hake. (Histological material from S. R. Goldberg, Whittier College, Whittier, CA.

c) Black skipjack. Histological material from K. Schaefer, IATTC, La Jolla, CA.

d) Pacific sardine from Peru about 24 h after spawning. Histological material from S. R. Goldberg, Whittier College, Whittier, CA.

below). These methods are useful, however, for estimating the duration of the spawning season, and they could possibly be used to provide a size or age-specific index of the relative duration of the season. At present these methodologies alone cannot be used to accurately identify in multiple spawners either postovulatory females or postspawning females, but it is possible that the GSI or maturity scale could be calibrated using histological detection of spawning frequency. Such a calibration would be worthwhile where a long historical record of GSI or maturity-scale determinations existed, because little is known of the interannual variation in spawning frequency in marine fish stocks. The technique of estimating spawning frequency using anatomical detection of females with hydrated ovaries is discussed in the next section.

Hydrated Oocyte Method

The incidence of females with hydrated oocytes can provide a quantitative estimate of the frequency of spawning in natural populations. Using this technique, DeMartini and Fountain (1981) found that the percentage of female queenfish, *Seriphus politus* (Family *Sciaenidae*), spawning per day averaged 13.6% over a 4-5 mo spawning season indicating that the mean interval between spawnings was 7.4 d. The chief advantage of the hydrated oocyte method is that spawning frequency estimates can be made using gross anatomical examinations of ovaries, and extensive histological processing and analysis are not required. Two preliminary steps required for estimation of spawning frequency using this method are outlined below.

1. Verification of gross anatomical criteria—The anatomical criteria used to identify hydrated ovaries must be verified using standard histological criteria, although advanced hydrated ovaries are relatively easy to identify from anatomical criteria or their weight (see Hunter et al. 1985; DeMartini and Fountain 1981). In addition, it is important to examine histologically the hydrated ovaries from females captured over the entire hydration period to determine the range of stages of hydration that are anatomically detectable.

2. Estimation of the optimal time for sampling—To estimate spawning frequency using the incidence of females with hydrated ovaries, females must be sampled prior to the onset of spawning, but at a time sufficiently close to the onset of spawning, so that all females destined to spawn are detectable using gross anatomical characteristics of the ovary. In addition, the duration of the period of hydration must be established and it must be documented that nearly all hydrated oocytes are spawned in less than 24 h. These determinations require that a series of samples of females be taken at regular intervals at sea over 24-48 h (see DeMartini and Fountain 1981). The optimal time for sampling females with hydrated ovaries can be determined from this series and biases caused by the onset of spawning or by the failure to detect hydration can be avoided.

The chief advantage of the hydrated ovary method over the use of the postovulatory follicles is that histological examination is not necessary after the preliminary work outlined above. On the other hand, our data on northern anchovy indicate three actual or possible disadvantages. First, sampling for the incidence of females with hydrated ovaries can be done only during a very limited portion of each day. In anchovy this period is probably 6 h or less, whereas females with day-old postovulatory follicles can be sampled at any time of day. The second possible disadvantage is that females with hydrated ovaries may be more vulnerable to trawls and perhaps other fishing gear than females in other reproductive states. This may bias an estimate based on hydrated ovaries. We find that spawning frequency estimates based on the number of female northern anchovy with hydrated ovaries and new postovulatory follicles can as much as double those estimates based on the incidence of day-old postovulatory follicles. However, this bias may be the result of the trawl oversampling the depth strata where hydrated females are abundant rather than a lack of avoidance by hydrated females. The third disadvantage of the hydrated ovary method is that incidence of females with hydrated ovaries is contagiously distributed among fish samples whereas females with day-old postovulatory follicles are not (Hunter and Goldberg 1980; DeMartini and Fountain 1981). This increases the variances of the estimate and demands a larger sample size. The contagion is probably caused by the separation of fish prior to spawning into groups composed mostly of males and hydrated females and other groups with fewer males and non-hydrated females.

Despite its actual and possible disadvantages, the simplicity of the hydrated ovary method is appealing. It seems a useful initial approach, especially since past estimates of spawning frequency were based on various speculative arguments (number of modal groups of oocytes in the ovary, or standing stock of yolked oocytes) and were in error by a factor of ten or more. The hydrated ovary method certainly provides a useful first approximation of spawing frequency at a relatively low cost.

ATRESIA

In northern anchovy, as well as in most other seasonal spawning fishes, a low incidence of oocyte atresia (degeneration of oocytes) occurs throughout the spawning season but becomes marked as the spawning season closes and the remaining advanced oocytes in the ovary are resorbed. The incidence of atresia is not used directly in the estimation of spawning biomass using the egg production method, but it is important for several other reasons: 1) A general knowledge of atresia is required to age postovulatory follicles because some stages of follicular atresia are very similar in appearance to late-stage postovulatory follicles; 2) it is a key histological marker for the cessation of spawning, and as a consequence can be used to determine whether or not a cruise period is optimal for biomass estimation (estimates near the end of the season have less precision); 3) it is necessary for accurate estimates of size at first maturity; and 4) it is essential for separating immature females from those in postspawning condition, a distinction required by the egg production method.

Atretic Stages

Oocyte degeneration or oocyte atresia has been divided into four or more sequential stages. We use the nomenclature and general characteristics defined by Bretschneider and Duyvene deWit (1947) and Lambert (1970a), but details of the descriptions of individual stages are based on our examination of northern anchovy ovaries (Hunter and Macewicz 1985).

During the initial stage of the atretic process, alpha (α), the entire oocyte is resorbed, including the yolk if present, by the hypertrophying granulosa cells of the follicle. In the second stage, beta (β), the major degeneration and resorption of the follicle (granulosa and thecal cells) occurs. In the third, gamma (γ), and fourth, delta (δ), atretic stages, regression of the theca and granulosa cells continues, greatly reducing the size of the follicle, and a yellow-brown

pigment appears in H & E sections. The histological characteristics used to identify these stages in northern anchovy are outlined below.

Alpha stage atresia—In the alpha stage of atresia the oocyte is resorbed, leaving only the follicular layers. The early phase of alpha stage atresia is characterized by disintegration of the nucleus, evident by an irregular shape and granular, dark, basophilic staining, and the disintegration of some of the yolk globules, indicated by less refractive globules, fused globules, or globules expanded and of less regular shape (Fig. 6a, b, c). The zona radiata slowly dissolves as indicated by the loss of striations and uneven diameter (Fig. 6b). In the subsequent phase of alpha atresia, granulosa cells enlarge and upon rupture of the zona radiata invade the degenerating oocyte (Fig. 6d). Yolk adjacent to the invading granulosa cells liquifies (loses all structural integrity and appears as a homogeneous eosinophilic area), and becomes phagocytized by the granulosa cells as indicated by the presence of yolk in the vacuoles of these cells. The basophilic staining cytoplasm is also resorbed by the granulosa cells. In the alpha stage of atresia, blood capillaries and vessels are numerous in the thecal connective layer which does not proliferate or invade the oocyte but remains as a thin layer covering the granulosa cells. The alpha stage ends when resorption of the oocyte is complete (all cytoplasm and yolk are gone). The resulting structure (beta stage) is usually much smaller than the original oocyte. The subsequent atretic stages (beta-delta) are steps in the resorption of the remaining follicle and the structure at this point is called an atretic follicle, the term atretic oocyte being reserved for only the alpha stage of atresia.

In unyolked oocytes, the alpha stage process is similar but without yolk (Fig. 6e, f). The nucleus disintegrates, the thin pre-zona radiata (if present) dissolves, and the granulosa cells enlarge and with only a slight proliferation phagocytize the unyolked oocyte. When resorption is complete, all that remains is the atretic follicle.

Beta stage atresia—Initially the beta-stage atretic follicle is a compact structure composed of numerous disorganized granulosa cells surrounded by a thin thecal and blood vessel layer. The nucleus of some of the granulosa cells is pycnotic and many of the cells contain intracellular vacuole(s) that may be empty or contain amorphous particles. Occasionally one or more large intercellular cavities may exist among the granulosa cells (Fig. 7b, d). Preovulatory beta-stage atretic follicles containing such cavities may easily be confused with postovulatory follicles (older than 48 h), and as a consequence we do not age postovulatory follicles older than 48 h (Hunter and Goldberg 1980). In addition, small (older) beta-stage atretic follicles from yolked oocytes (Fig. 7c, d) are indistinguishable from beta-stage atretic follicles from unyolked oocytes. Thus, we do not identify the original oocyte type undergoing atresia in beta or subsequent atretic stages; such distinctions are made only for alpha-stage atretic oocytes.

Three different patterns of atresia may occur at the conclusion of the beta stage: 1) The follicle may follow the classic pattern outlined by Bretschneider and Duyvene deWit (1947) and pass through subsequent gamma and delta stages (both characterized by increased pigmentation, see below); 2) the follicle may be completely resorbed during the beta stage, leaving no histological characteristics that can be identified; and 3) the follicle may pass directly from a beta stage structure to a delta structure without passing through the intervening gamma stage. In northern anchovy, either the duration of the gamma stage is very short or few follicles pass through the gamma stage into the delta stage, because in regressing ovaries the incidence of gamma stages is very low compared to those of either beta or delta stages.

Gamma stage atresia—The gamma-stage atretic follicle is usually much smaller than the typical beta stage follicle (Fig. 7e). The granulosa cells contain flocculent material of light-yellow hue, and have nuclei of very irregular shape. The granulosa cells are surrounded by many fewer thecal cells and blood vessels than occur in the beta-stage atretic follicles. Occasionally we see an atretic follicle of quite different appearance in anchovy ovaries which we classify as a gamma-stage atretic follicle; they are included in the gamma stage because they also contain flocculent material of light-yellow hue. In this case, the flocculent yellow material is extracellular rather than intercellular and the material is encapsulated by a layer of granulosa and thecal cells. It is possible that the extracellular flocculent material is produced by the disintegration of granulosa cells.

Delta stage atresia—The diagnostic characteristic of this stage is the presence of a dark yellow-brown, finely granular pigment in the granulosa cells (Fig. 7f). The delta-stage atretic follicles are normally very small structures typically composed of 2-20 granulosa cells in the connective tissue stroma. Thecal cells and blood vessels no longer directly encompass the granulosa cells because they have been absorbed into the ovarian connective tissue stroma.

Atretic States

For population work it may be impractical to grade every ovary for the abundance of each of the four atretic stages. To simplify the assessment of atretic condition of the ovary, we have defined four atretic states which are based on some of the atretic stages described in the previous section. The duration of these states was estimated for anchovy in the laboratory and then assessed using starvation to trigger resorption of the ovary (Hunter and Macewicz 1985). In addition, the spawning potential of sea-caught females that were classed in these states was estimated by calculating the incidence of females with postovulatory follicles and hydrated eggs within each atretic state ($N > 5000$); their results are summarized below.

Atretic state 0—Females classed as atretic state 0 have yolked oocytes present and no alpha atresia of yolked oocytes. Beta stage atresia may be present but is not considered, as it cannot be separated with certainty from late-stage postovulatory follicles (>48 h old). Female anchovy in this state have a high potential of spawning, with spawning occurring at a frequency of every week to 10 d.

Atretic state 1—Females with yolked oocytes in which <50% of the yolked oocytes are in the alpha stage of atresia. Frequency of spawning of females classed in this state is less than half of that in females of atretic state 0. Thus, atretic state 1 is an index of a decline in spawning rate (Table 1). Females appeared to persist in this state under natural conditions for extended and probably variable periods, but the state persisted in the laboratory under induced starvation for only 5-7 d. Atretic state 1 is the most common atretic condition during peak spawning periods, and it can be used to detect differences in spawning between length classes; it appears to be a more sensitive index of differences in reproductive rate among length classes than is the incidence of postovulatory follicles (Hunter and Macewicz 1985).

Atretic state 2—Females with yolked oocytes in which ≥50% of the yolked oocytes are in the alpha stage of atresia. Females in atretic state 2 persisted for about 9 d in the laboratory, and it seems to have a similarly short duration in natural populations. It is the best measure of the absolute rates of ovary resorption in the population

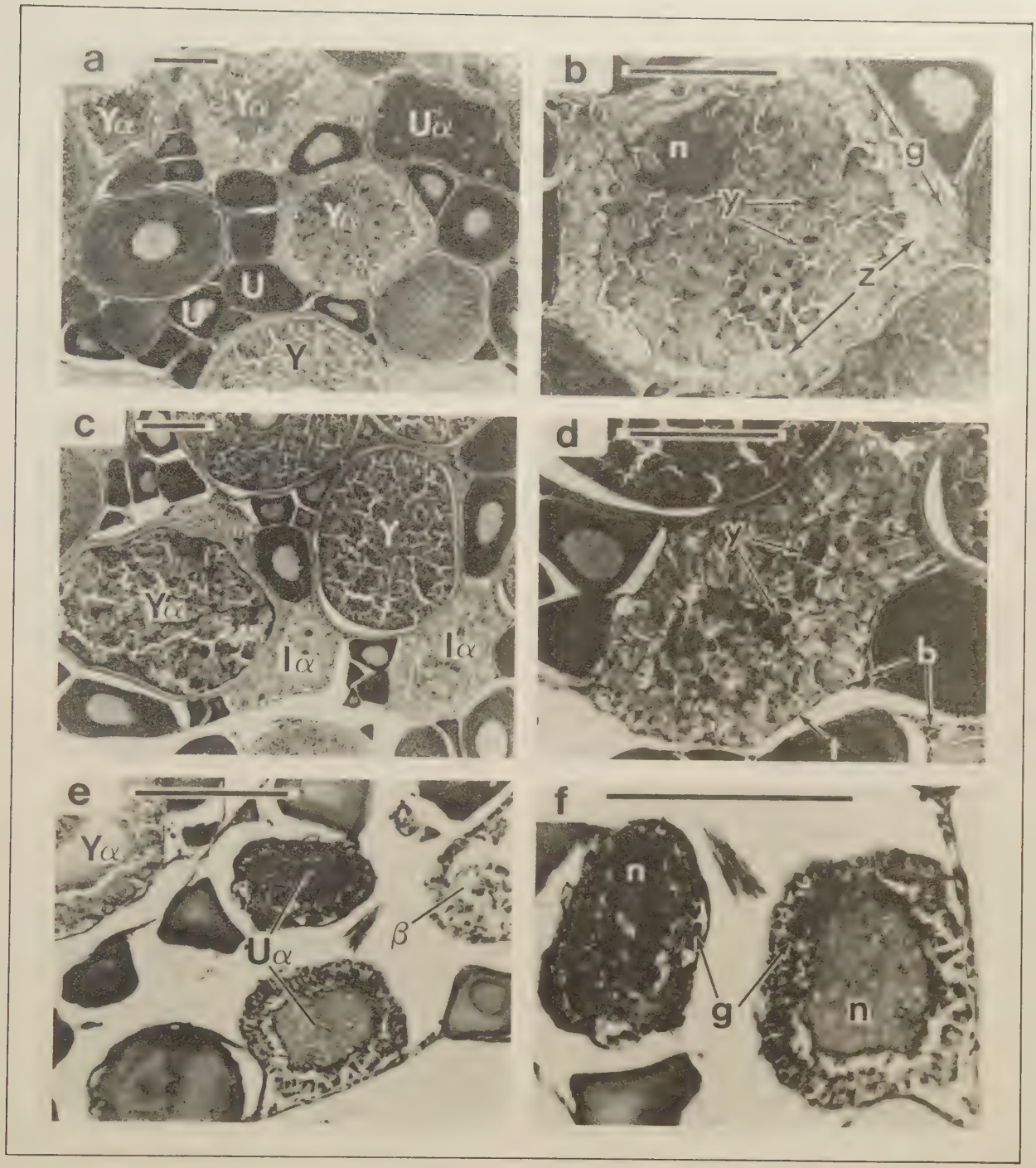

Figure 6.—Alpha (α) stage atresia in yolked (Y) and unyolked (U) oocytes. Bar = 0.1 mm.

a, b) Yolked oocyte undergoing alpha atresia ($Y\alpha$). Notice dark irregular nucleus (n), uneven dissolving zona radiata (z), hypertrophic granulosa cells (g); and alpha atresia of a large unyolked oocyte ($U\alpha$).

c, d) Only remnants of yolk material (y) remain among the invasive phagocytizing granulosa cells in this late phase of alpha atresia ($I\alpha$). Note also the thecal layer (t) and the closely associated red blood cells (b). Y = yolked oocyte; $Y\alpha$ = alpha yolked atretic oocyte.

e, f) Unyolked oocytes in the alpha stage of atresia ($U\alpha$). Note enlargement of granulosa (g) and disintegration of nucleus (n). $Y\alpha$ = alpha yolked atretic oocyte; β = beta atretic follicle.

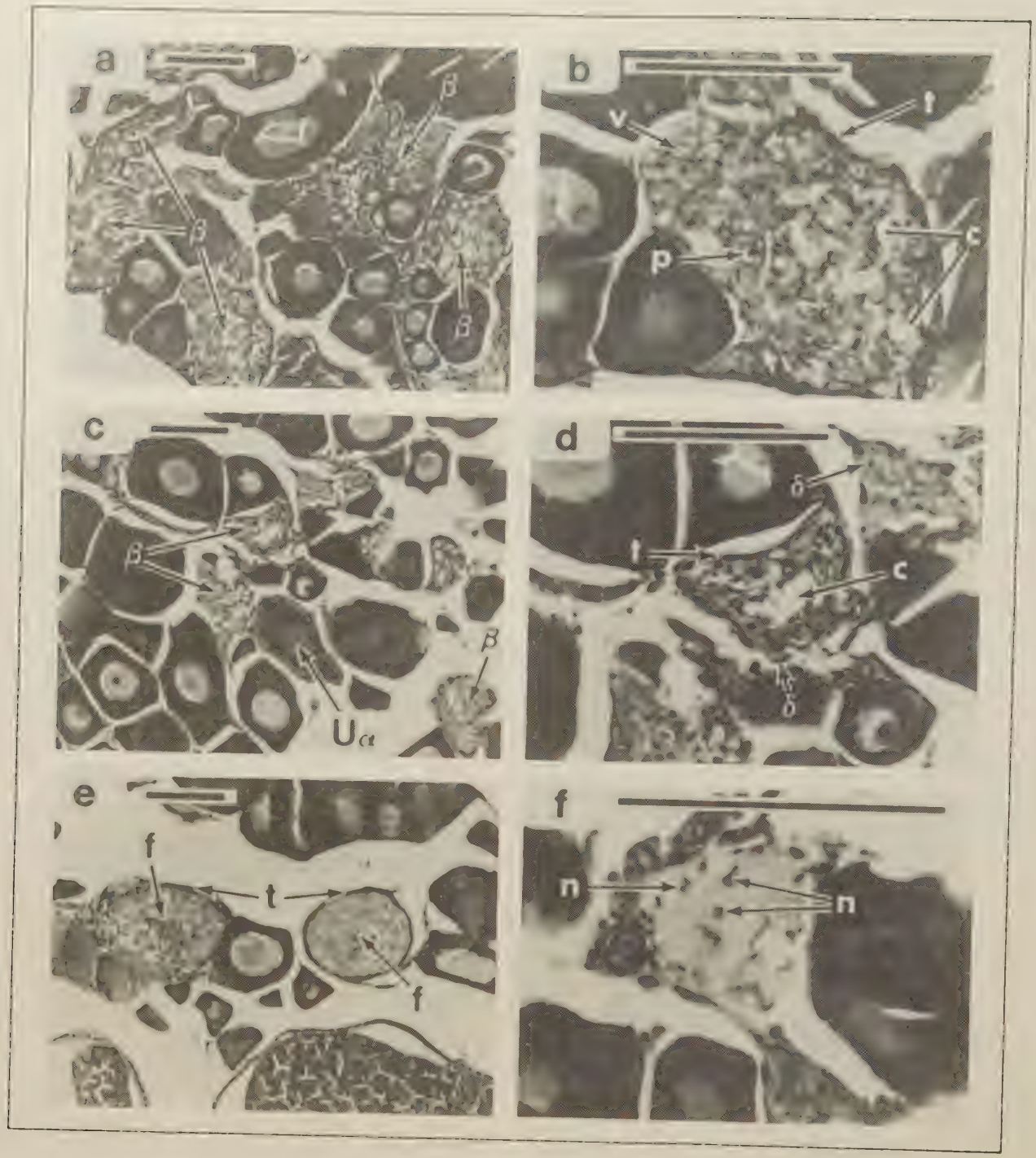

Figure 7.—Stages of atresia following complete yolk absorption. Bar = 0.1 mm.

a, b) Typical beta (β) stage atresia. Note the disorganized granulosa cells with some pycnotic nuclei (p) or intracellular vacuoles (v). t = thecal connective cell layer; c = intercellular cavities.

c, d) Disintegration of granulosa continues in these older beta (β) atretic follicles. Note the large intercellular cavity (c) and the prominent, contracted thecal cell layer (t). Also present is an unyolked oocyte in early alpha ($U\alpha$) stage and several delta (δ) stage atresia cells.

e) Two types of gamma (γ) atresia seen in northern anchovy ovaries. Note flocculent material (f) and the thecal connective cell layer (t).

f) Delta (δ) stage atresia characterized by dark-yellow, fine, granular pigment and an irregular nucleus (n).

Table 1.—Histological classifications[1] of northern anchovy ovaries and the reproductive implications. PO = postovulatory.

Ovary	State	Key histological characteristics	Reproductive implications
Spawning States			
Active	Hydrated	Hydrated oocytes present	Spawning will occur in >12 h[2].
	Age 0-day PO	Postovulatory follicles <24 h old.	Spawning occurred <24 h ago[3].
	Age 1-day PO	Postovulatory follicles 24-48 h old.	Spawning occurred 1 d ago[3].
Atretic States			
Active	0	Yolked oocytes present; no alpha-stage atretic yolked oocytes.	High probability of spawning within 1-10 d[4].
	1	Alpha stage atresia of yolked oocytes; ≥50% of yolked oocytes are affected.	Probability of spawning is half that of atretic state 0[5].
Inactive	2	Alpha stage atresia of yolked oocytes; ≥50% of yolked oocytes are affected.	Probability of spawning = 0; may have had an active ovary 1-20 d ago (average 8 d)[6].
	3	No yolked oocytes present; beta stage atresia present.	Probability of spawning = 0; may have had an active ovary ≥16-30 d ago[6].
	Immature	No yolked oocytes present; no beta atretic follicles.	Probability of spawning = 0; no evidence of past reproduction within last 30 or more d. May develop an active ovary within ≥31 d[7].

[1]Ovaries are classed into both spawning and atretic states, but the reproductive implications are for a single-state classification and not for a combination of atretic and spawning states.

[2]Hunter and Macewicz 1980.

[3]Hunter and Goldberg 1980.

[4]In peak spawning months the average female spawns at 1-10 d intervals (Hunter and Leong 1981); hence females with active ovaries are likely to spawn within 1-10 d depending on maturity of the ovary.

[5]Based on the fraction of females in spawning states within atretic state 1 (Hunter and Macewicz 1985).

[6]Hunter and Macewicz 1985.

[7]Hunter and Leong 1981.

and could be used to forecast the end of reproduction near the end of the spawning season. A high incidence of females in this state indicates that cessation of spawning in the population is imminent.

Atretic state 3—Females without yolked oocytes in which beta stage atresia is present. This state identifies females in late postspawning condition. The state persisted for about 30 d in the laboratory, and it may last much longer under natural conditions while the numerous small oocytes are resorbed. (The laboratory data indicate that the duration of this state could be increased if definitions were redefined to include gamma + delta stages of atresia which have a longer life in the ovary than does the beta stage.) This state is used to separate females in postspawning condition from immature females (females with no previous reproductive history). Consequently, identification of females in this state is essential for accurate estimates of age or size at first maturity and for an accurate definition of spawning biomass using the egg production method.

TESTING THE ASSUMPTION OF SEASONALLY DETERMINATE FECUNDITY

A critical assumption underlying some biomass estimates from ichthyoplankton data and many estimates of annual fecundity in fishes is that the annual fecundity in a species is determined at the beginning of the spawning season. Validation of this assumption of determinate fecundity requires proof that 1) all oocytes destined to be spawned in a season are identifiable at the beginning of the season and no new spawning batches are recruited from the reservoir of small unyolked oocytes that exist in the ovary the year round, and 2) the identified standing stock of yolked oocytes that constitute the maximum potential annual fecundity are in fact spawned and only a negligible quantity of these oocytes are resorbed at the end of the season.

The traditional evidence for determinate fecundity is the presence of a major gap in oocyte maturity stages or size classes between the oocytes matured for the season and the reservoir of immature oocytes present year-round in the ovary (Yamamoto 1956). The presence of such a gap in oocyte classes in females taken at the beginning of the season seems to be adequate proof that the standing stock of oocytes is a measure of maximum annual fecundity, as long as the gap is not between a batch of hydrated oocytes and other yolked oocytes. The absence of such a discontinuity in oocyte classes is evidence for indeterminate fecundity. In some cases, however, determinate annual fecundity is believed to exist despite the fact that oocyte size classes are continuously distributed in the ovary. In such cases, spawning frequency and batch fecundity for the species must be estimated over the season and their product (fecundity × frequency) compared to the standing stock of mature oocytes at the beginning of the season. This was done for whiting and haddock in the laboratory by Hislop (1975) and Hislop et al. (1978).

For northern anchovy, Hunter and Leong (1981) compared field estimates of spawning frequency with standing stocks of oocytes. They showed that oocytes as small as 0.1 mm must be included in the annual fecundity to account for 10 spawnings per yr and estimated that anchovy spawn an average of 20 or more times per yr. Their analysis clearly documents the existence of indeterminate fecundity in anchovy. However, anchovy have a continuous oocyte distribution, and so indeterminate fecundity is expected in this species.

In species for which determinate fecundity is inferred because of a discontinous oocyte distribution, it is also necessary to document that the maximum annual fecundity estimated from the standing stock of oocytes is in fact realized in the sea (see above). To validate this assumption requires an analysis of rates of atresia of yolked and partially yolked oocytes. The key atretic state in such an analysis would be one similar to our atretic state 2 where many oocytes are in the alpha stage of atresia. It should be borne in mind that this state may have a short duration.

In anchovy the mean duration of this state is only 9 d and the maximum only 20 d (Hunter amd Macewicz 1985). Consequently, sampling to determine the extent of resorption of oocytes must be done over a relatively short period near the end of the season; otherwise, significant oocyte resorption may not be detected. Substantial oocyte resorption has been observed in the Pacific hake, *Merluccius productus*, which seems to have a discontinuous oocyte distribution (Foucher and Beamish 1977). They conclude that the standing stock of yolked oocytes is very likely in excess of the number of oocytes that will be spawned, except in exceptional years.

In conclusion, an assumption of determinate fecundity requires extensive work to prove, and in some cases may be only wishful

thinking. The most conservative assumption is that seasonal fecundity is indeterminate for multiple spawning fishes and that estimates of batch fecundity and spawning frequency are required. The documented cases of determinate fecundity appear to be restricted to boreal or cold temperate climates where spawning seasons are short. Thus in most of the world's oceans indeterminate fecundity and multiple spawning are the rule for epipelagic spawners, and estimates of spawning frequency are essential both for biomass estimation using ichthyoplankton data and for estimates of annual fecundity.

FUTURE APPLICATIONS OF HISTOLOGICAL CLASSIFICATION OF OVARIES

An important future application of the histological classifications we have described is the study of reproductive processes in multiple spawning fish populations. This work requires not only histological classification but sufficient laboratory calibration and analysis of sea data to be able to specify the reproductive implications and duration of each class. Sufficient data exist on northern anchovy to make a relatively accurate interpretation of the reproductive significance of each spawning and atretic state (Table 1). Reproductive state and its approximate duration can now be related to the physiological state of the female (age, size, fat content, biochemical composition, instantaneous growth rate from otoliths or RNA/DNA ratios, and environmental conditions). In this way it may now be possible to identify the factors controlling the 2-3 fold variation in batch fecundity (Hunter et al. 1985), the potentially large variation in spawning frequency and duration of the spawning season, and, most importantly, the functions regulating partitioning of energy between reproduction and growth.

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Comparison between Egg Production Method and Larval Census Method for Fish Biomass Assessment

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ABSTRACT

Ichthyoplankton abundance and production are related to the aggregate weight of spawning adults. The problem is first formulated in terms of annual production and fecundity. The abundance of northern anchovy larvae, used to index annual production, is related to the daily production of eggs. Advantages of the egg production method of assessing stock biomass are discussed.

INTRODUCTION

This final article discusses the general problem of relating ichthyoplankton data to the aggregate weight of spawning adults and reviews various solutions. The first section deals with the problem as traditionally formulated; that is, the annual production of eggs and larvae is related to adult biomass by annual fecundity. The second section reformulates the problem in instantaneous terms; that is, daily production and daily fecundity. The third section relates the two ichthyoplankton methods used to estimate the northern anchovy populations: The larval census method and the egg production method. The fourth section discusses the advantages of the egg production method.

ANNUAL PRODUCTION OF EGGS AND LARVAE

Although ichthyoplankton surveys have been conducted for many purposes—to detect unexploited fish stocks, to study development, biogeography and systematics of fishes, and to forecast future population growth—Hensen and Apstein (1897) initially proposed that the surveys be used to index the spawning population of adults. The idea was appealing because of the ease of sampling eggs and larvae relative to sampling adults. Ichthyoplankters are found in a restricted vertical range of the sea, they have no or very limited ability to avoid capture, and simple sampling gear can be employed which allows for increase in the density of observations.

The basic model relating the weight of the spawning population to production of eggs is (Saville 1964)

$$B = \frac{P}{F \times R}$$

where B is the weight of the spawning population, P is the annual production of eggs, F is the annual female fecundity on a weight basis (annual specific fecundity), and R is the female fraction of the population. Application of the method requires the ability to identify reproductive products of the target species and knowledge of their development rates, the geographic and seasonal extent of spawning, and the determination of batch size and number of batches spawned by a female each year.

The production of eggs can be estimated from egg abundance adjusted for their development time and the mortality suffered during this period (Sette 1943). Annual abundance is estimated by integrating individual observations over both the survey area and the spawning season. Several spatial integration procedures have been employed: Contouring the observations and measuring the topographic volume (Buchanan-Wollaston 1926; Van Cleve and Seymore 1953; Simpson 1959); summing of strata averages raised by strata areas (Sette 1943; Smith 1972; Berrien et al. 1981); and summing individual observations raised by the area that each observation represents (Sette and Ahlstrom 1948). The three methods yielded similar results when applied to surveys of Pacific sardine spawning by Sette and Ahlstrom (1948). Temporal integration has been accomplished by summing successive surveys weighted by the portion of the year that they represent (Sette and Ahlstrom 1948; Berrien et al. 1981) by measuring the area under the curve that describes the survey abundance by time of year (Simpson 1959; Bannister et al. 1974), integrating a normal curve scaled to describe the seasonal variation in spawning activity (Saville 1956), and summing quarterly estimates of average abundance (Smith 1972). English

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(1964) advocated procedures which averaged over space and time because of the large (and unpredictable) variance between observations; he argued that estimates from integrating procedures did not have reliable estimates of variability and were not amenable to comparisons between years. Adjustments for development time and mortality can be made either before integration (e.g., Berrien et al. 1981), after integration (Sette 1943), or in a stepwise mixture of integration and adjustment (e.g., Bannister et al. 1974). Unless there is a strong correlation between abundance and rate of development or mortality within the survey area, the order of calculation should make little difference in the final estimate of annual egg production. If these factors are independent, then the various procedures discussed above are algebraically equivalent.

The most recent statistical advance in the description of ichthyoplankton surveys is the use of a weighted negative binomial distribution to describe the sample distribution (Zweifel and Smith 1981). Individual observations can be weighted for sampling bias and error (e.g., extrusion, avoidance, variations in the sample volume, and development rate), and the mean of the fitted distribution is an unbiased estimate of the production rate with a well-described error structure. Production is estimated by raising this mean over the space-and-time stratum encompassed by the observations. By fitting a weighted negative binomial to successive stage-specific sample distributions, an age-specific production curve may be derived (Hewitt 1982). Application of this procedure to the 30-yr time series of northern anchovy larvae surveys revealed a change in the microdistribution pattern (patch dispersal) of larvae as they grew and a change in the mortality rate with the level of egg production (Hewitt 1982).

Annual specific fecundity is estimated from knowledge of the number of eggs released per unit weight per spawning and the number of spawnings per year. Both factors can be affected by environmental conditions such as the amount, quality, and availability of food. For boreal species, for which spawning seasons tend to be distinct, short, and predictable, estimating annual specific fecundity may be less of a problem than for temperate and tropical populations, with protracted or even continuous spawning seasons. The problem of estimating annual specific fecundity may be sidestepped by assuming that production or abundance of eggs and larvae is proportional to the weight of the adult population; the proportionality constant is estimated by regressing the abundance of larvae on an independent estimate of population size (Smith 1972; Saville and Schnack 1981). Thus

$$B = k \times C \qquad \text{(Smith 1972)}$$

where C is the annual census of larvae and k is the proportionality constant. This procedure has come to be known as the Larval Census Method (Smith and Richardson 1977). Application of the method assumes that annual specific fecundity does not vary between years nor does the larval mortality rate (hence the use of larval abundance rather than production). To the extent that specific fecundity and larval mortality rate are independent of population size, violation of the assumptions introduces error but not bias in the estimate of the constant of proportionality.

The theory and conduct of ichthyoplankton surveys are discussed thoroughly in Smith and Richardson (1977); their bibliography (Smith and Richardson 1979) contains over 1,000 citations and is complete through 1973, including proceedings of the first Symposium on the Early Life History of Fish held at Oban, Scotland in 1973 (Blaxter 1974). The estimation of adult spawning stock size from egg and larval surveys is also discussed by Saville (1964, 1977), Hempel (1973), and Ulltang (1977). The proceedings of both symposia on the Early Life History of Fish (Blaxter 1974; Lasker and Sherman 1981) include several reports of estimates of stock size from ichthyoplankton surveys and provide a good introduction to a much larger body of literature.

DAILY PRODUCTION OF EGGS AND LARVAE

Development of the ability to determine the near-term reproductive status of a female allowed the stock-estimation problem to be reformulated in terms of parameters that were easier to measure. Hunter and Goldberg (1980) and Hunter and Macewicz (1980) developed criteria to describe the anchovy spawning cycle apparent in gonad tissue with a resolution of one day; i.e., it is possible to classify fish that were spawning on the day of capture, those that spawned the day previous to capture, those that spawned 2 days previous to capture, etc. Thus a direct measure of daily specific fecundity is possible as the product of the fraction of females spawning per day times the weight-specific batch fecundity. It is not necessary to integrate egg production over the spawning season because both fecundity and production can be expressed on a daily basis. The model relating the weight of the population to the daily production of eggs is (Parker 1980)

$$B = \frac{P_O}{F_s \times R}$$

where B is the weight of the spawning population, P_O is the daily production of eggs, F_s is the specific daily fecundity, and R is the female portion of the population. These parameters can be estimated from a representative sample obtained over the spawning habitat. For a fish that spawns continuously over a protracted spawning season (e.g., anchovy), the observations need not be made synoptically; rather, data collected over a 30-day cruise can be pooled without introducing large errors. This method has been used since 1980 to estimate the spawning stock of northern anchovy (Picquelle and Hewitt 1983a, b).

The production of eggs was estimated from the age-specific production curve (Fig. 1). The production rate of eggs at time zero (spawning) was raised over the survey area to estimate daily production by the population. The variance of this estimate was reduced using postsurvey stratification of the survey area.

Specific daily fecundity was estimated as the product of three parameters:

$$F_s = \frac{F \times S}{W}$$

where F is the average batch fecundity, S is the average fraction of adult females spawning per day, and W is the average weight of an adult female. These parameters were estimated from a sample of adult fish obtained concurrently with a sample of their eggs. The adult sample consists of ~100 trawl stations, and the egg sample ~1,000 plankton stations. Results of the four surveys conducted through 1983 are summarized in Table 1.

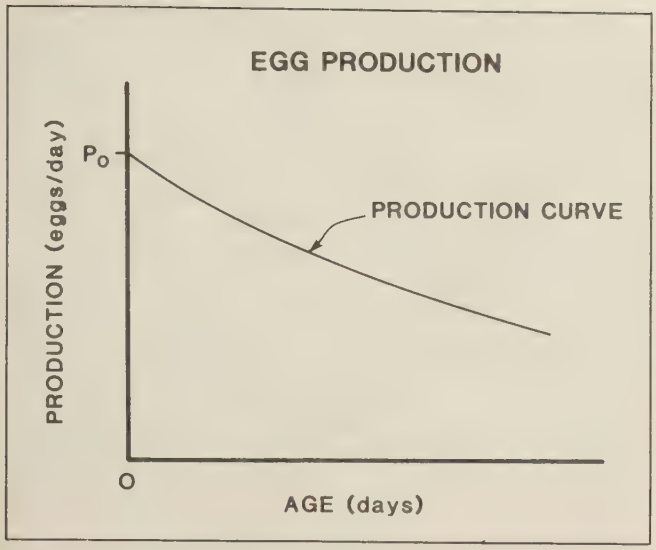

Figure 1.—Age-specific production curve of fish eggs. The curve is estimated from abundance-at-age data and the intercept is defined as the production rate (per unit area) of eggs at time zero (spawning).

RELATIONSHIP BETWEEN EGG PRODUCTION AND LARVAL CENSUS

The standing stock of larvae results from the production of eggs modified by mortality. Eggs are spawned continually by the adult population; some of them hatch, some of the larvae survive to metamorphosis, and many more perish as eggs or larvae. The average abundance of eggs and larvae is the sum of this process over all ages; i.e., the area under the production curve (Fig. 2). Thus, egg production is proportional to the zero intercept of the production curve, and the larval census is proportional to the area below the production curve. Both are raised above the survey area for population estimates and have a constant relationship to the extent that mortality is constant. The annual northern anchovy larval census was the latter value summed over four quarters (Smith 1972).

The expected abundance of northern anchovy larvae is the integral of the larval production curve from the time of hatch to 30 days (the oldest age at which anchovy larvae are effectively caught). The larval production curve is an extension of the egg production curve, and this is the basis for an analytical connection between the two methods. The larval production curve may be parameterized as (Lo 1985; Hewitt and Brewer 1983)

$$P_t = P_h \left(\frac{t_h}{t}\right)^{\beta} \quad \text{for } t < t_h$$

where P_t is the production of t-day old larvae, t_h is the age at hatch, and β is the larval mortality coefficient which describes how rapidly survival improves with age. The egg production curve may be parameterized as (Picquelle and Hewitt 1983a)

$$P_t = P_O e^{-Zt}$$

Table 1.—Egg production estimates of northern anchovy spawning biomass (1980-83). C.V. = coefficient of variation (standard deviation/mean).

		1980[1,4]		1981 (Feb)[1,4]		1981 (Apr)[1,4]		1982[2,4]		1983[3,5]	
		Est.	C.V.	Est.	C.V.	Est.	C.V.	Est.	C.V.	Est.	C.V.
Daily egg production ($\times 10^{12}$ eggs/d)	P_O	26.34	0.111	20.96	0.101	12.59	0.087	13.51	0.237	17.25	
Fraction of mature females spawning/d	S	0.142	0.125	0.106	0.122	0.125	0.092	0.120	0.038	0.094	
Sex ratio of weight (females/total)	R	0.478	0.120	0.501	0.063	0.495	0.051	0.472	0.081	0.549	
Batch fecundity (no. eggs/batch)	F	7,751	0.075	8,329	0.052	8,846	0.045	10,845	0.047	5,297	
Average female weight ($\times 10^{-6}$ t)	W	17.44	0.055	13.37	0.039	16.20	0.029	18.83	0.019	11.20	
Daily population fecundity ($\times 10^{-6}$ eggs/day-ton)	$q=SRF/W$	30.28		33.03		33.84		32.53		24.35	
Spawning biomass	$B=P_O/q$	870	0.225	635	0.183	372	0.147	415	0.257	652	0.211

[1]Documented in Stauffer, G.D., and S. J. Picquelle (Unpubl. manuscr.) Egg production estimates of spawning biomass of the northern anchovy, *Engraulis mordax*, for 1980 and 1981. SWFC, La Jolla, CA.

[2]Documented in Picquelle and Hewitt (1983a).

[3]Documented in Picquelle and Hewitt (1983b).

[4]These surveys were conducted with a 333-μm mesh net estimated to retain 91% of the eggs. Original estimates were elevated by a factor of 1/0.91.

[5]Biomass estimate was actually calculated as the sum of three regional biomass estimates. Parameter estimates reported here are averaged over the entire survey area for comparison purposes. See Picquelle and Hewitt (1983b) for regional parameter estimates.

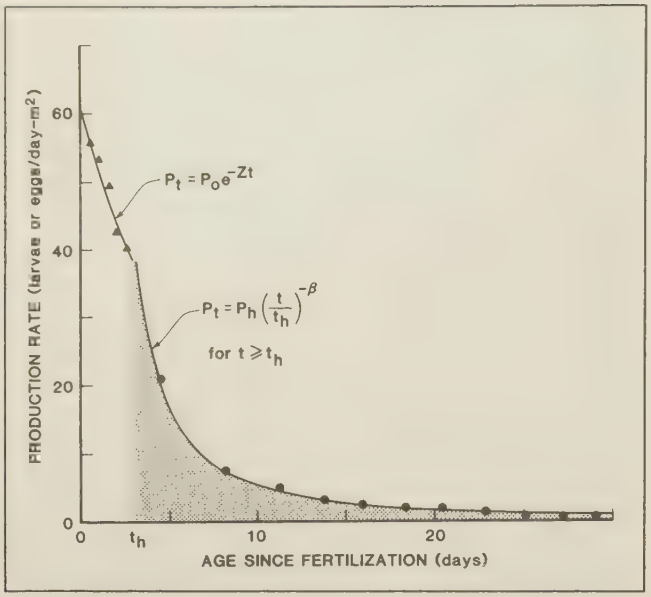

Figure 2.—Age-specific production curve of anchovy eggs and larvae. The average abundance of larvae is the shaded area under the larval production curve.

where Z is the instantaneous mortality coefficient, assumed to be constant over the incubation period. The production of eggs at the end of the incubation period may be substituted for the production of larvae at t_h.

The annual census of larvae averaged 2.12 times the standing stock during the spawning season (Stauffer and Picquelle 1980). The annual larval census also underestimated the true abundance of larvae because of losses due to extrusion and avoidance (Smith 1972; Lenarz 1972, 1973; Lo 1983). Thus, the annual census of larvae may be related to the daily production of eggs:

$$\hat{C} = 2.12(r)\, P_O e^{-Zt} \left(\frac{t_h}{\beta - 1}\right)(1 - (t_h/30)^{\beta-1})$$

where r is the fraction of the larval population represented in an ichthyoplankton sample; r is a variable which is dependent on the size distribution of the larvae caught and the time of their capture. These relationships are developed in more detail by Stauffer (1983), Picquelle and Hewitt (1983a, b), and Lo (1985).

As was noted above, spawning biomass may be estimated using the larval census method by multiplying C times the constant of proportionality; this assumes no variability in reproductive output or early mortality. Spawning biomass may be estimated using the egg production method by dividing P_O by the daily specific fecundity; this procedure specifically accounts for variability in reproductive output and early mortality.

Results from the time series of egg production are listed below, together with projected larval census estimates. During the first two years larval surveys were conducted concurrently, and the measured larval census agreed well with the projected larval census.

YEAR	P_O ($\times 10^{12}$ eggs/d)	C ($\times 10^9$ larvae)	C ($\times 10^9$ larvae)
1980	26.34	17.22	18.10
1981 (Feb.)	20.96	35.12	28.58
(Apr.)	12.59	30.85	
1982	13.51	20.97	
1983	17.25	15.80	

The egg production surveys were conducted with the CalVET sampler described in Smith et al. (1985). The CalVET sampler has a 25-cm diameter mouth and is retrieved vertically to the surface from 70 m depth; it was designed to sample a small volume of water in a short period of time relative to the standard CalCOFI bongo sampler used to conduct larval surveys. The bongo sampler is a paired net with 60-cm diameter mouths and is retrieved obliquely to the surface from 210 m depth. Although the sampling gears are quite different, they yield consistent and compatible estimates of ichthyoplankton production.

These relationships may also be used to estimate historical egg production from the production curve of larvae and the abundance of eggs (Lo 1985). MacCall and Methot (1983) assembled the various measures of anchovy spawning biomass (egg production, back-projected egg production, acoustic surveys, and aerial spotter logs); they used acoustic and aerial data to fill in missing ichthyoplankton survey years and scaled the measures to the recent (1980-82) egg production estimates of biomass. The resulting 30-yr time series is the basis for a population model used to manage the harvest of anchovy (Pacific Fishery Management Council 1983).

ADVANTAGES OF THE EGG PRODUCTION METHOD

The egg production method is based on a model which uses instantaneous rates (daily egg production and daily specific fecundity). As such, a great economy is realized relative to annual estimates, because the necessary field data can be obtained on a single cruise. In the case of anchovy, at least four surveys were required to define the annual spawning activity (Smith 1972). Thus a major advantage to the egg production method is the relatively low cost of collecting field data.

The egg production method is also more precise than other ichthyoplankton methods. Early mortality and adult reproductive output are both measured rather than assumed or extrapolated from other observations. The extrapolated estimates may be accurate but they have a finite error associated with the estimation process that is eliminated when these parameters are measured directly. In the case of anchovy, the egg production method is also probably more accurate than the larval census method (Picquelle and Hewitt 1983a). This is because of inaccuracies in estimating the proportionality constant between the annual census of larvae and the weight of the adult stocks.

The egg production estimate of spawning biomass can be broken down into several factors each of which can be estimated, with an associated variance, from a sample of the population. These variances, together with the covariance between the parameters, may be used to estimate the variance of the estimate of spawning biomass. This is not so easily done with estimates based on annual rates because certain factors must be estimated without an idea of their variance (e.g., the number of batches spawned per year). It is also possible to measure the real variance of the egg production estimate of spawning biomass by conducting multiple surveys during the same year as was done in 1981.

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